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Health Assessment Document for Trichloroethylene

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16. ABSTRACT

Trichloroethylene (TCI) is a solvent widely used in the industrial degreasing of metals. It has been detected in the ambient air of a variety of urban and non-urban areas of the United States and also, in natural and municipal waters.

The weight of available evidence obtained from both animal and human data suggest that long-term exposure to environmental levels of TCI poses no serious health concern to the general population. No teratogenic potential has been demonstrated for TCI in studies conducted to date with experimental animals. With respect to the mutagenic potential of TCI, the data on pure TCI do not allow a conclusion to be drawn. If TCI is mutagenic, the available data suggest it would be a very weak, indirect mutagen. Based on available animal cancer data, the classification of TCI under the criteria of the International Agency for Research on Cancer (IARC) could either be "sufficient" or "limited." Because there are no adequate epidemiologic data, the overall ranking of TCI would place it in a category in which it would be a probable human carcinogen or one that cannot be classified as to its carcinogenicity.

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DISCLAIMER

This report is an external draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

The Office of Health and Environmental Assessment, in consultation with an Agency work group, has prepared this health assessment to serve as a "source document" for EPA use. Originally the health assessment was developed for use by the Office of Air Quality Planning and Standards, however, at the request of the Agency Work Group on Solvents, the Assessment scope was expanded to address multimedia aspects.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses are placed in perspective with observed environmental levels.

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The Environmental Mutagen Information Center (EMIC) in Oak Ridge, Tennessee, kindly identified literature bearing on the mutagenicity of TCI. Their initial report and subsequent updates were used to obtain papers from which the mutagenicity assessment was written.

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1. SUMMARY AND CONCLUSIONS

Trichloroethylene (1,1,2-trichloroethylene) (TCI) is a solvent widely used in the industrial degreasing of metals. It has no known natural sources. Current U.S. production is estimated at about 130,000 metric tons per year. Of the trichloroethylene used in the United States, 80 to 95 percent evaporates to the atmosphere. In addition to the workplace, TCI is found in a variety of urban and nonurban areas of the United States and other regions of the world. It has been measured in ambient air and water. An average ambient air concentration of about 1 part per billion (ppb) would be expected for some large urban centers. Concentrations as high as 32 ppb have been measured in urban centers in the United States, and 47 ppb has been measured in urban Tokyo. Ambient air concentrations of TCI are greatly influenced by the rate and geographic distribution of emissions and the rate of decomposition and transposition in the atmosphere. The average mixing ratio in the troposphere of the northern hemisphere is 11 to 17 parts per trillion (ppt). Reaction with hydroxyl radicals is the principal mechanism by which TCI is scavenged from the atmosphere.

Trichloroethylene has been detected in both natural and municipal waters in the United States. Up to 403 ppb has been measured in some surface and subsurface waters, and, in finished drinking water, concentrations have ranged from 1 to as much as 32 ppb. There is no direct evidence of bioaccumulation of TCI in the food chain. Few studies have been made of the ecological consequences of TCI in the environment.

The pharmacokinetics and metabolism of TCI have been studied in man as well as in animals. As with other related halogenated aliphatic solvents, the principal route of exposure to TCI is by inhalation of vapor in air followed by lung absorption. Ingestion of drinking water contaminated with TCI is a secondary source of exposure. Both exposure routes are important because TCI contaminates air, water and food. Ingested TCI enters the portal system first, perfusing the liver: thus, at low concentrations only a small proportion of TCI would enter the general circulation, because uptake and metabolism by the liver are expected to be nearly complete. In contrast, inhaled TCI directly enters the systemic circulation and is widely distributed in the body.

Because of its lipophilic nature, TCI readily crosses lipid-containing cell membranes and portions selectively in adipose tissue upon chronic or long-term exposures to even low ambient air concentrations, until steady-state is reached. Physical activity increases uptake.

The amount of TCI absorbed after oral ingestion is virtually complete; with air exposure, the amount of TCI absorbed increases in proportion to its concentration in inspired air, the respiratory rate, and duration of exposure. TCI is eliminated by two major processes, pulmonary excretion of unchanged TCI and liver metabolism to urinary metabolites. At air concentrations of 500 ppm or less, humans are estimated to metabolize between 60 to 90 percent of absorbed TCI. There is no evidence that TCI metabolism in man is saturation-dependent, at least up to about 300 ppm. Studies have not been made of metabolism after oral exposure in man; however, an even higher proportion of an absorbed amount of TCI is expected to be metabolized following exposure by this route. The principal identified urinary metabolites of TCI are trichloroethanol (TCE) and its glucuronide, and trichloroacetic acid (TCA). The evidence is strong that, in the liver, TCI is first metabolized to a reactive epoxide (trichloroethylene oxide) by a microsomal P_{450} system. This reactive epoxide covalently binds extensively to cellular macromolecules. The epoxide is further converted to chloral hydrate and thence to TCE and TCA. Metabolism of TCI is enhanced by drugs and xenobiotics such as barbiturates, oral antidiabetic agents, and PCBs, which induce the hepatic microsomal P_{450} metabolizing system. Heightened toxicity can result from clinical drug interactions known to occur with ethanol, barbiturates, disulfiram and Warfarin.

Toxicity testing in experimental animals, coupled with limited human data derived principally from overexposure situations, suggests that long-term exposure of humans to environmental (ambient) levels of TCI is not likely to represent a health concern. Limited controlled human exposures indicate that impairment of the performance of certain mental and physical tasks may occur during acute exposure to levels in the range of 100 to 200 ppm. There is no information that such impairment, representative of dysfunction of the central nervous system (CNS), would or would not occur during chronic, low-level exposures. At levels found or expected in the ambient environment, such an effect would be unlikely. Similarly, dysfunction of the liver or kidneys would not be likely during environmental exposures. Damage to renal tubules and signs of liver dysfunction have been observed only in experimental animals during exposures to excessively high levels (>1000 ppm).

Teratogenicity is another health endpoint for which available data from experimental animals suggest that the conceptus is not uniquely susceptible to

TCI. Exposure of rats, mice, and rabbits, during gestation, to levels (300 ppm) greatly in excess of those generally found in the environment has not been observed to result in any teratogenic effects. It should be noted, however, that such results cannot be easily extrapolated to humans. The teratogenic potential of TCI for humans is still unknown and represents a basis for continued awareness and investigation.

With respect to the mutagenic potential of TCI, available data provide suggestive evidence that commercial-grade TCI is a weakly active indirect mutagen, causing effects in a number of different test systems representing a wide evolutionary range of organisms. Based on this, commercial TCI may have the potential to cause weak or borderline increases above the spontaneous level of mutagenic effects in exposed human tissue. The observations that commercial TCI causes adverse effects in the testes of mice suggest TCI may cause adverse testicular effects in man too, provided that the pharmacokinetics of TCI in humans also result in its distribution to the gonads. The data on pure TCI do not allow a conclusion to be drawn about its mutagenic potential. However, mutagenic potential cannot be ruled out. If it is mutagenic, the available data suggest that TCI would be a very weak, indirect mutagen.

Trichloroethylene, both in epoxide-stabilized form and in epoxide-free form, has been shown to be positive for the induction of malignant tumors of the liver in both male and female B6C3F1 mice during lifetime bioassay under the auspices of the National Toxicology Program. This constitutes a signal that TCI might be a carcinogen for humans. It should be recognized, however, that there is a substantial body of opinion in the scientific community to the effect that the mouse liver overreacts to chlorinated organic compounds, in contrast to the rat, and that the induction of liver cancer in the mouse represents only a promoting action for spontaneous liver tumors, which normally occur with substantial incidence. Furthermore, this promoting action might be related to liver damage associated only with high-level exposures to chlorinated agents such as TCI. However, the evidence is inconclusive either for this restrictive position on the mouse liver carcinogenic response to chlorinated organics, or for the position that the mouse liver is as good as any other mammalian indicator of carcinogenicity for these compounds.

Based on available animal cancer bioassay data, the classification of the TCI results under the criteria of the International Agency for Research on Cancer (IARC) could be either "sufficient or "limited," depending on the differing current scientific views about the induction of liver tumors in mice by chlorinated organic compounds. Because there are no adequate epidemiological data in humans, the overall ranking of TCI under the criteria of IARC could be either Group 2B or Group 3, i.e., the more conservative scientific sentiment would regard TCI as a probable human carcinogen, but there is considerable scientific sentiment for regarding TCI as an agent that cannot be classified as to its carcinogenicity for humans.

RECOMMENDATIONS FOR FURTHER STUDIES

Areas in which incomplete information is available, and that should be considered in formulating research needs, are presented below. The needs listed, however, are not necessarily in the order of relative priority.

1. Teratogenicity and Reproductive Effects. There are few animal studies via inhalation that adequately assess the teratogenic and reproductive effects potential of TCI. Uncertainty about these biological endpoints in humans should serve as a stimulus for future research.

2. Neurobehavioral Toxicity. These have been few animal studies of the effects of TCI on the nervous system and behavior. Most endpoints studied have been relative insensitive. Further studies are warranted on more sensitive endpoints.

2. INTRODUCTION

Trichloroethylene (TCI) is a member of a family of unsaturated chlorinated aliphatic compounds. Trichloroethylene, though a water and solid waste contaminant, is primarily of interest in situations involving ambient air exposure. It is released into ambient air as a result of evaporative losses during production, storage, and/or use. It has no known natural sources. It is photochemically reactive in the troposphere and is removed by scavenging mechanisms, principally via hydroxyl radicals.

The scientific data base concerning the effects of TCI on humans is limited. Effects on humans have generally been ascertained from studies involving individuals occupationally or accidentally exposed. During such exposures, the concentrations associated with adverse effects on human health were either unknown or far in excess of concentrations found or expected in ambient air. Controlled TCI exposure studies have been directed toward elucidating the effects on the CNS, effects on clinical chemistries, and pharmacokinetic parameters of TCI exposure.

Since epidemiologic studies have not been able to assess adequately the overall impact of TCI on human health, it has been necessary to rely greatly on animal studies to derive indications of potential harmful effects. Although animal data cannot always be extrapolated to humans, indications of probable or likely effects in animal species increase confidence that similar effects may occur in humans.

This document is intended to provide an evaluation of the scientific data base concerning TCI. The literature has been comprehensively reviewed through September 1983. The publications cited in this document represent a majority of the known scientific references to TCI. Reports which had little or no bearing on the issues discussed were not cited. Some topic areas, such as effects on terrestrial ecosystems, are only briefly discussed. Such topics reflect the nature of the scientific data base.

3. BACKGROUND INFORMATION

3.1 PHYSICAL AND CHEMICAL PROPERTIES

Trichloroethylene (TCI) is a colorless, highly volatile liquid that is miscible with a variety of solvents. Some of its important physical properties are shown in Table 3-1.

TABLE 3-1. PHYSICAL PROPERTIES OF TRICHLOROETHYLENE

Molecular weight	131.39
Boiling point	87°C
Vapor pressure	94 mm Hg @ 30°C
Vapor specific gravity	4.55 at boiling point (air = 1)
Solubility @ 20°C	0.107 gm percent

At standard temperature and pressure (25°C, 1 atmosphere), one part-per-million (ppm) of the vapor is equivalent to 5.38 mg/m^3.

Trichloroethylene is photochemically reactive and autooxidizes upon catalysis by free radicals (McNeill, 1979). Autooxidation is greatly accelerated by high temperatures and exposure to ultraviolet radiation (McNeill, 1979). Some of its degradation products, e.g., hydrochloric acid (HCl), are corrosive to metals. These decomposition products include dichloroacetyl chloride, phosgene, carbon monoxide, hexachlorobutene, and HCl (McNeill, 1979.)

Decomposition is catalyzed when TCI comes in contact with aluminum metal. The HCl produced reacts with aluminum to yield aluminum chloride ($AlCl_3$) which catalyzes formation of hexachlorobutene (McNeill, 1979). Sufficient quantities of aluminum have been reported to cause violent decomposition of TCI (Metz and Roedy, 1949). While TCI is nonflammable under ordinary conditions, mixtures of TCI and oxygen will ignite at temperatures above 25.5°C when the TCI concentration is between 10.3 and 64.5 percent (Jones and Scott, 1942).

To inhibit its decomposition, stabilizers are added to commercial formulations of TCI (McNeill, 1979). Commonly used stabilizers in vapor degreasing grades of TCI are neutral inhibitors and free radical scavengers (McNeill, 1979). Epoxides (including epichlorohydrin, a known carcinogen) are added to scavenge free HCl or $AlCl_3$. Antioxidants are usually added in quantities less than 1 percent by weight (Hardie, 1964). Fuller (1976) reported that inhibited grades of TCI are stable up to 130°C in the presence of air, moisture, light, and common construction metals. At higher temperatures, inhibitors are less effective and corrosion of metals can result. Stabilizers that have been used in TCI formulations are shown in Table 3-2.

TABLE 3-2. STABILIZERS IN TRICHLOROETHYLENE FORMULATIONS (Beckers, 1972; U.S. EPA, 1975; Hardie, cited in: Kirk Othmer, 1975; Schlossburg, 1980)

Amyl alcohol
Propanol
Diethylamine
Triethylamine
Dipropylamine
Diisopropylamine
Diethanolamine
Morpholine
N-Methylmorpholine
Aniline
Acetone
Ethyl acetate
Borate esters
Ethylene oxide
1,2 Propylene oxide
1,2 Epoxy butene
Cyclohexene oxide
Propylene oxide
Butadiene dioxide
Styrene oxide
Pentene oxide
2,3 Epoxy 1-propenol
3, Methoxy-1,2 epoxy propane
3, Ethoxy-1,2 epoxy
Stearates
2-Methyl-1,2 epoxy propanol
Epoxy cyclopentanol
Epichlorohydrin
Tetrahydrofuran
Tetrahydropyran
1,4-Dioxane
Dioxalane
Trioxane
Alkoxyaldehyde hydrazones
Methylethyl ketone
Nitromethanes
Nitropropanes
Phenol
o-Cresol
Thymol
p-tert-Butylphenol
p-tert-Amylphenol
Isoeuganol
Pyrrole
n-Methyl pyrrole
n-Ethyl pyrrole
(2-pyrryl) Trimethylsilane
Glycidyl acetate
Isocyanates
Thiazoles

Trichloroethylene decomposes at temperatures up to 700°C. Substances found in the decomposition mixture include dichloroethylene, tetrachloroethylene, carbon tetrachloride, chloroform, and methyl chloride (Hardie, 1964). Formation of phosgene, a highly toxic gas, was observed when TCI came in contact with iron, copper, zinc, or aluminum over the temperature range 250°C to 600°C (Sjöberg, 1952). When TCI came in contact with zinc at 450°C, 69 mg phosgene per gram of TCI was produced.

Trichloroethylene is not readily hydrolyzed by water (McNeill, 1979). In the presence of strong alkali (e.g., sodium hydroxide) TCI can decompose to dichloroacetylene, an explosive, flammable, and highly toxic compound (Humphrey and McClelland, 1944). Noweir et al. (1973) recommended that TCI be stored in cans or dark glass bottles to minimize decomposition.

Because of its lipophilic properties, TCI can be expected to partition selectively in the body's adipose tissue. The relationships between such partitioning and manifestation of its toxic properties is discussed in Chapter 4. The unsymmetrical nature of the molecule and the electron-withdrawing effect of the chlorine substituents (leading to destabilization of the charge at the double bond) have been proposed as factors responsible for the supposed mutagenicity of TCI (Bonse and Henschler, 1976).

Measurements of the lipophilic potential of TCI have been made by a number of investigators (Waters et al., 1977; Mapleson, 1963; Powell, 1947; Sato and Nakajima, 1979). Mapleson (1963) reported an oil/water partition coefficient of 960:1 at 37°C. Waters et al. (1977) reported the oil/water coefficient as 900:1. Recently Sato and Nakajima (1979) reported an olive oil/water partition coefficient of 552:1 at 37°C and an olive oil/blood partition coefficient of 76:1. The relationship of TCI to other compounds with respect to partition coefficients is shown in Table 3-3 (Sato and Nakajima, 1979).

3.2 ENVIRONMENTAL FATE AND TRANSPORT

3.2.1 Production

Trichloroethylene has been declining in production in recent years owing to restrictions on emissions (Federal Register, 1977), substitution by other solvents, and other factors. According to statistics published by the U.S. International Trade Commission (1980), 133,000 metric tons of TCI were produced in 1980.

TABLE 3-3. PARTITION COEFFICIENTS OF TRICHLOROETHYLENE AND OTHER CHLORINATED HYDROCARBONS (Sato and Nakajima, 1979)

Solvent	Oil/water	Oil/blood	Log P (partition coefficient)
Dichloromethane	21	16	1.32
Chloroform	115	39	2.06
Carbon tetrachloride	1444	150	3.16
1,2-Dichloroethane (EDC)	40	23	1.60
Methyl chloroform	383	108	2.58
Tetrachloroethylene	4458	146	3.65
Trichloroethylene	552	76	2.74

Recent estimates of world production of TCI are not available. Such figures for 1973 indicated 700,000 metric tons were produced (McConnell et al., 1975). About two-thirds was produced outside the United States.

In 1977, TCI was produced by five American manufacturers (SRI, 1978). The producers and production capacities are shown in Table 3-4.

TABLE 3-4. U.S. PRODUCTION CAPACITY FOR TRICHLOROETHYLENE (SRI, 1978)

Producer	Site	Annual capacity (metric tons)
PPG Industries	Lake Charles, LA	127,000
Dow Chemical	Freeport, TX	68,040
Diamond Shamrock	Deer Park, TX	24,948
Occidental Petroleum	Taft, LA	22,680
Ethyl Corporation	Baton Rouge, LA	18,144

Most of the TCI produced commercially is derived from ethylene or dichloroethane (EDC) (McNeill, 1979). Dichloroethane, produced by chlorination of ethylene, can be further chlorinated to TCI and tetrachloroethylene at 280^{o} to 450^{o}C. Catalysts include potassium chloride, aluminum chloride, fuller's earth, graphite, activated carbon, and activated charcoal (McNeill, 1979). Maximum conversion to TCI (75% of EDC feed) is achieved at a chlorine-to-EDC ratio of 1.7:1 (McNeill, 1979).

Oxychlorination of ethylene or EDC to TCI is achieved at 425^{o}C and at 20 to 30 psi (McNeill, 1979). Common catalysts are mixtures of potassium and cupric chlorides. Conversion efficiency is between 85 and 90 percent.

3.2.2 Use

It has been estimated that from 80 to 95 percent of the TCI produced in the United States is used in the degreasing of fabricated metal parts (SRI, 1978; McNeill, 1979; Chemical and Engineering News, 1975). The remaining 5 to 20 percent is divided equally between exports and miscellaneous applications. Use in vapor degreasing is estimated to account for 87 percent of production, according to industry trade statistics (Chemical Marketing Reporter, 1978).

In addition to its use as a vapor degreasing agent, TCI has a wide variety of other uses (Table 3-5). About 4,500 to 6,800 metric tons are consumed annually in the production of polyvinyl chloride, in which TCI is used as a chain-transfer agent (McNeill, 1979).

TABLE 3-5. MISCELLANEOUS USES OF TRICHLOROETHYLENE
(McNeill, 1979; Waters et al., 1977)

Adhesive formulations
Paint-stripping formulations
Low-temperature heat-transfer medium
Carrier solvent in industrial paint systems
Solvent in textile dyeing and finishing

Uses of TCI that have been discontinued in the United States because of environmental and health restrictions include use as an inhalation anesthetic (Page and Arthur, 1978; Waters et al., 1977), use in fumigant mixtures (Farm Chemicals Handbook, 1976), and use as an extractant in the decaffeination of coffee. Use in anesthetic procedures appears to be continuing to some extent in other countries (Cundy, 1976).

Declining consumption of TCI from the early 1970's has been offset by the increased use of substitute solvent, e.g., methyl chloroform and methylene chloride (U.S. EPA, 1979).

3.2.3 Emissions

Because of its dispersive use pattern in degreasing operations, most of the TCI used is expected to be emitted to the atmosphere. There are no known natural sources of TCI. Graedel and Allara (1976) concluded that atmospheric chemical production of TCI was negligible. Recent emissions estimates (U.S. EPA, 1979) for 1976, pertaining to vapor degreasing, cold cleaning, and miscellaneous use categories suggest that 130,000 metric tons were discharged to the atmosphere.

Solvent emissions from vapor degreasing occur primarily during unloading and loading of the degreaser (Bucon et al., 1978). Daily emissions of a single spray degreasing booth may vary from a few pounds to 1,300 pounds (Bucon et al., 1978). In uncontrolled degreasing operations, as much as 2.8 kg/h can be emitted, based on 200,000 pounds of metal cleaned per day (Bucon et al., 1978).

McConnell et al. (1975) estimated that, for 1973, global emissions of TCI amounted to about 1 million metric tons.

Singh et al. (1979) estimated global emissions of TCI for 1977 at 435,000 metric tons ± 50 percent. Estimates were derived from production figures and usage patterns.

3.2.4 Persistence

Many processes occur in the troposphere which can alter the atmospheric levels of TCI. Once emitted into the troposphere, vertical and horizontal mixing occurs. Transport is highly dependent upon the length of time TCI remains in the troposphere, which is determined largely by the extent to which TCI reacts with hydroxy free radicals (·OH), the principal scavenging mechanism for TCI and many other halogenated compounds in the atmosphere.

Edney et al. (1983), on the basis of the observed rate of reaction of TCI with hydroxy radicals in a reaction cell, calculated an atmospheric lifetime for TCI of about 54 h. A hydroxy concentration in the troposphere of 10^6 molecules/ cm^3 was assumed.

The average northern hemisphere background mixing ratio is between 11 and 17 ppt (Singh et al., 1979), which suggests that TCI is short-lived in the troposphere. Singh et al. (1979) estimated a residence time of about 2 weeks, an estimate based on a seasonally-averaged OH concentration of 4×10^5 molecules cm^{-3} and a rate constant (National Bureau of Standards, 1978), at 265^0K, of 2.30×10^{-12}. Singh and coworkers (1979) estimated that, given an ·OH concentration of 1×10^6 molecules cm^{-3}, 20 percent of the TCI in ambient air can be destroyed each day. Crutzen et al. (1978) estimated a residence time of 11 days for TCI. Derwent and Eggleton (1978) estimated the lifetime at 0.04 years (∿ 15 days). The percentage of the ground level injection of TCI that was estimated to survive free radical attack was 0.4 percent.

Seasonal variations in ·OH concentrations, important for longer-lived species, are not expected to play a significant role in the tropospheric survival of TCI. Altshuller (1980) calculated that in January (when ·OH levels and solar flux are low) 0.6 days would be required for the photochemical decomposition of 1 percent of the ambient TCI. In July (when ·OH levels and solar flux are high), only 0.07 days is required.

Trichloroethylene was observed to have a slow decomposition rate in dilute aqueous solution (Dilling et al., 1975). Its half-life (in the dark) was 10.7 months at ambient temperatures. When the solution was exposed to sunlight, 75 percent of the TCI decomposed in 12 months, compared with 54 percent for the reaction in the dark. A correction for the amount of TCI that volatilized into the air space above the solution was not employed. On the other hand, Pearson and McConnell (1975), in their determinations of the hydrolytic decomposition, extrapolated to zero volatilization. Their estimate was a half-life of 30 months.

The major route of removal of TCI from water is volatilization. Volatilization of TCI from water has been investigated by several authors. Dilling et al. (1975) have shown that the loss of TCI from an agitated dilute aqueous solution occurs exponentially with an evaporative half-life of 21 ± 3 min. Scherb (1978) measured the volatilization of TCI from an aeration channel in a wastewater treatment plant and found the half-life to be 3 h. The rate of volatilization of TCI from surface waters in the environment has been reported by Zoeteman et al. (1980). TCI was found to have a half-life of 1 to 4 days in the Rhine River and 30 to 40 days in a tidal estuary. Smith et al. (1980) have shown that the rate of volatilization of TCI and other low-molecular-weight compounds from various bodies of waters is a function of reaeration rates. From the work of Smith et al. (1980), it is estimated that the half-life for TCI in surface waters ranges from 3 h, for rapidly moving shallow streams, to 10 days or longer for ponds and lakes (Table 3-6).

TABLE 3-6. ESTIMATED HALF-LIFE OF TCI IN SURFACE WATERS

Water Type	TCI half-life (days)
Pond	11
Lake	4 to 12
River	1 to 12

Source: Calculated from information in Smith et al. (1980).

3.2.5 Degradation

The transformation products of the decay of TCI have been investigated in chamber studies simulating atmospheric conditions. Two principal transformation products have been observed, phosgene and dichloroacetyl chloride. Dahlberg (1969) observed that both were produced when TCI was photooxidized in air. Gay et al. (1976) also found formyl chloride in addition to phosgene and dichloroacetyl chloride, when TCI was photooxidized in the presence of nitrogen dioxide (NO_2). A maximum observed consumption rate for TCI of 70 percent per hour was reported. In this system, dichloroacetyl chloride, identified by long-path infrared spectroscopy, was the principal product. In a smog chamber system containing 2.5 ppm TCI and 0.5 ppm NO_2, TCI disappeared at the rate of about 25 percent per hour (Dimitriades et al., 1983). Ozone built up to 0.5 ppm. The presence of equal amounts of tetrachloroethylene and TCI in a chamber containing NO_2 did not alter the consumption rate of TCI above TCI controls.

Edney et al. (1983) observed a number of degradation products when hydroxy radicals reacted with TCI in a 700 liter cell coupled to an FTIR spectrometer and scanning interferometer. Reaction products observed were phosgene, dichloroacetyl chloride, formyl chloride, carbon monoxide, and nitric acid. The rate constant of reaction was 3.6×10^{-12} cm^3/sec.

Lillian et al. (1976), finding phosgene formation from the photooxidation of the TCI congener, tetrachloroethylene, considered that phosgene formation also was likely from other chloroethylenes. Singh (1978) reported that ·OH-catalyzed photodecomposition of TCI, as occurs in the troposphere, can result in phosgene as the principal transformation product.

Under simulated atmospheric conditions in the presence of nitric oxide (NO) and nitrogen dioxide (NO_2), TCI (10 ppm) was very short-lived (less than 3.5 h) (Dilling et al., 1976). A consumption rate up to 30 percent per hour was reported. The high reactivity of TCI and its catalytic role in the formation of photochemical smog have been recently discussed (U.S. EPA, 1978a; National Academy of Sciences, 1977). Free radicals (e.g., $\cdot RO_2$), are formed when TCI is photodecomposed, and these in turn react with NO by oxidizing it to NO_2. According to the following reaction sequence, NO_2 results in the formation of ozone (O_3):

$$(1)\quad NO_2 \xrightarrow{hv} NO + O$$

$$(2)\quad O + O_2 + M \text{ (third body)} \longrightarrow O_3 + M$$

Because chamber studies simulate some atmospheric reactions believed to occur in the environment, further studies of greater complexity would be needed to determine the extent and pathways by which TCI is photodecomposed. Phosgene and dichloroacetyl chloride, though likely to be formed in the troposphere, also are subject to transformation and scavenging processes.

3.3 LEVELS OF EXPOSURE

3.3.1 Analytical Methodology

Of the analytical methods and procedures available for detecting and quantitating halogenated hydrocarbons present in ambient air, water, soil, foods, etc., two systems are commonly used: gas chromatography/mass spectrometry (GC-MS) and gas chromatography with electron capture detection (GC-EC). Each system has the capability of detecting TCI concentrations as low as a few parts-per-trillion by volume. The usefulness of GC-EC is that the apparatus can be used in the field to provide quasi-continuous measurements by intermittent sampling (every 15 to 20 minutes). Such use can circumvent decomposition that results when samples of TCI are stored for long periods before assay (Pellizzari, 1974; Lovelock, 1974b).

3.3.1.1 Gas Chromatography with Electron Capture Detection--The GC-EC methodology has been reviewed by Pellizzari (1974) and by Lovelock (1974b). The EC detector is specific in that halogenated compounds are quantified while non-halogenated hydrocarbons are not detected. Thus, high background levels of non-halogenated hydrocarbons in ambient air or water samples do not interfere with measurements of halogenated compounds. In a complex mixture in which several compounds may have similar retention times, alteration of the operating parameters of the GC-EC system will usually provide separation of the components.

In the method developed by Rasmussen et al. (1977), the detection limit for TCI was 0.3 ppt. With four samples of ambient air, the percent standard deviation was 23.0 by electronic area integration (+ 5 percent by peak height measurement). The system, which employs a freezeout concentration loop, has the capability of measuring various halocarbons in 500-ml aliquots of air. Total analysis time is 40 min. The GC column consisted of a 6-mm x 3-m stainless steel column packed with 10 percent SF-96 on 100/120 mesh-Chromosorb W.

A similar analytical procedure was employed by Harsch and Cronn (1978) to measure levels of halocarbons in 30-ml samples of low-pressure stratospheric air. For TCI, the detection limit was 1 ppt and the precision (percent standard deviation) for 10 samples with a mixing ratio of 22 ppt was 10 percent.

Gas chromatography with electron capture detection also has been the method of choice by Singh and coworkers for measuring the ambient air levels of a wide variety of halocarbons. Singh et al. (1979) maintained the EC detector at 325°C. The GC column used was 6-ft x 1/8-in. stainless steel, containing 20 percent SP2100, 0.1 percent Carbowax 1500 on 100/120 mesh Supelcoport. Singh et al. (1979b) recommended two columns for separation of TCI: (a) silicone oil 10 percent DC 200 on 30/60 mesh Chromosorb W in a 6-ft x 1/4-in. stainless steel column or (b) 20 percent SP-2100 on 80/100 mesh Supelcoport in a 15-foot x 1/8-inch column.

The applicability of GC-EC to analysis of TCI and other halohydrocarbons purged from water samples has been discussed by Bellar et al. (1979). Preconcentration on a Tenax and silica gel trap was recommended.

Singh et al. (1979a) avoided the use of Tenax traps for preconcentrating organics in air samples because of artifacts. They thought that oxygen or ozone was oxidizing the Tenax monomer and producing electron-absorbing oxygenated species, thus interfering with EC detection of the organics of interest.

Makide et al. (1980) used GC, coupled with constant current ECD, to measure TCI in ambient air at sites in Japan. The reported detention limit was < 0.2 ppt (< 1.36×10^{-6} mg/m^3). Sampling canisters were made of stainless steel and were polished electrochemically. Preconcentration was carried out on a silicone OV-101 column at -40°C. Column temperature was raised at a rate of 5°C per minute up to 70°C for separation of components.

A direct headspace technique for use with EC detection has been reported by Piet and coworkers (1978). This technique avoids the need for preconcentration traps when water samples are purged. An overall detection limit below 1 µg/l was reported. With isothermal operation of the GC, a detection limit for TCI of 0.05 µg/l was reported. An OV225 capillary column was used.

Dietz and Singley (1979) recently described a headspace method applicable to EC detection of volatile organics from water samples. A standard deviation of 5 percent was reported for routine analyses of drinking, natural, and industrial waters. Concentrations ranging from 0.1 ppb to several ppm of TCI were detected. In this method, the water sample is sealed in a vial until organics equilibrate between the water and vial headspace. A major advantage is that only relatively volatile, water-insoluble compounds tend to partition in the headspace. In a comparison of this method with the purge and trap technique, Dietz and Singley (1979) obtained comparable results. However, the chromatograms from the purge-trap method were of poorer quality. The headspace method was recommended for routine sample analyses of volatile halohydrocarbons.

3.3.1.2 Gas Chromatography-Mass Spectrometry--While GC-EC relies on retention time and ionization efficiency for compound identification, the equally sensitive GC-MS system identifies compounds by their characteristic mass spectra. However, the expense, bulk, and demands on the operator preclude routine use of GC-MS in field measurements. Often, GC-MS is used to verify results obtained by GC-EC.

Comparisons between the precision afforded by GC-MS *versus* GC-EC have been made by Cronn and co-workers (1976). In these comparisons, the detection limit was defined based on a peak height three times the noise level with a 75-ml sample loop. With TCI at an average mixing ratio of 128 ppt, the detection limit (GC-MS) was 23 ppt (with 3 doped samples of zero air). The percent standard deviation was 10. More recent data from Cronn and Harsch (1979) show a slightly lower detection limit (16 ppt) for a 100-ml freezeout sample. The sensitivity of the GC-MS technique was achieved using direct coupling of the gas chromatograph and mass spectrometer and selective ion monitoring (at mass 95 for TCI).

Pellizzari et al. (1976) used Tenax GC to absorb TCI and other halogenated hydrocarbons from ambient air. Recovery was made by thermal desorption and helium purging into a freezeout trap. The estimated detection limit when high resolution GC-MS is used is 1.92 ppt (0.01×10^3 mg/m^3). Accuracy was reported at ±30 percent. Included among the inherent analytical errors were (1) the ability to accurately determine the breakthrough volume, (2) the percent recovery from the sampling cartridge after a period of storage, and (3) the reproducibility of thermal desorption from the cartridge and its introduction into the analytical system. To minimize loss of sample, cartridge samplers should be enclosed in cartridge holders and placed in a second container that can be sealed, protected from light, and stored at 0°C. The advantages reported for Tenax include (1) low water retention, (2) high thermal stability, and (3) low background levels (Pellizzari, 1974; Pellizzari, 1975; Pellizzari, 1977; Pellizzari et al., 1976).

In an analytical system using Tenax GC as the absorbent, Krost et al. (1982) reported an estimated detection limit for TCI of 2 ppt. Analysis of the breakthrough volumes at various temperatures suggests that Tenax GC is not a particularly effective absorbent for TCI.

Resolution of TCI from other hydrocarbons was reported to be effective when Carbopak C-HT, a graphitized thermal carbon black treated with hydrogen at 1,000 °C was used as the adsorbent in a GC column (Knoll et al., 1979). Flame ionization was used in detection. Interference between TCI and ethylene dichloride was reported with Porapak T.

Gas chromatography-mass spectrometry has been more widely used in measurements of volatile organic compounds in aqueous samples. In this regard, gas purging followed by trapping on an absorbent has been extensively used in combination with GC-MS detection. A recent review of this system, as applied to TCI and other volatile organics, has been made by Bellar et al. (1979). A combination of Tenax GC (60/80 mesh) and silica gel was recommended. Purging was accomplished at a water temperature of about 22°C. In general, the method is applicable to compounds that have a low solubility in water and a vapor pressure greater than water at ambient temperatures. For well-defined samples when the probability of unexpected compounds is low, the EC detector could be used. The detection limit of this system was reported to be in the 0.1 to 1 μg/l range. Desorption onto the head of the column can be accomplished in one step by heating.

3.3.2 Calibration

Both static and dynamic procedures have been used to calibrate the instrumentation used for measurement of TCI. Because of the very low levels of TCI found or expected in ambient air, the generation of known low-level concentrations of TCI is critical in instrument calibration.

Singh and coworkers (1979, 1977) employed permeation tubes to calibrate their GC-EC instrumentation. A tube constructed of FEP Teflon (wall thickness of 0.03 in.) was found to be satisfactory for TCI. Errors in the permeation rate were less than 5 percent (Singh et al., 1979). It was considered the best, most accurate means of generating primary standards. Since primary standards could not be used in field operations, secondary standards were generated and compared to them. The permeation rate at the 95-percent confidence level was 246.0 ± 20.1 mg/min (Singh et al., 1977).

Primary and secondary standards prepared by multiple dilutions were used by Cronn and coworkers (1976, 1977a,b).

3.3.3 Sampling and Sources of Error

Common approaches used to sample ambient air for trace gas analysis are presented below (National Academy of Sciences, 1978).

(1) Pump-pressure samples: A mechanical pump is used to fill a stainless steel or glass container to a positive pressure relative to the surrounding atmosphere.

(2) Ambient pressure samples: An evacuated chamber is opened and allowed to fill until it has reached ambient pressure at the sampling location. If filling is conducted at high altitude, the container may easily become contaminated when returned to ground level.

(3) Adsorption on molecular sieves, activated charcoal, or other adsorbents.

(4) Cryogenic samples: Air is pumped into a container and liquefied, and a partial vacuum is created which allows more air to enter. This method allows for the collection of several thousand liters of air.

Singh and coworkers (1979) employed electropolished stainless steel vessels in their sampling of tropospheric air. Vessels were checked for background contamination until it was reduced to less than 2 to 3 percent of the expected background concentration of a given trace substance. Typically, vessels that maintained positive pressure were not affected by room contamination.

Sampling and storage of ambient air collected at stratospheric altitudes present more of a problem. Because of the low pressures in the sampling vessels, contamination may easily result when vessels are returned to ground level. Cronn and Robinson (1978) and Harsch and Cronn (1978) reported that contamination of low-pressure stainless steel vessels precluded use of data for TCI. Whole-air samples were collected at stratospheric altitudes. Contamination occurred during the sample-transfer procedure because of high mixing ratios of halocarbons in laboratory air.

To overcome contamination of low-pressure samples, Harsch and Cronn (1978) developed a low-pressure sample-transfer technique. A vacuum transfer procedure employing a freezeout loop (Rasmussen et al., 1977) was found to yield results with sufficient sensitivity and with minimal contamination problems. A leak-tight system was considered essential. System components were silver-soldered.

Sample collection and handling and preservation of water samples have been discussed by Kopfler et al. (1976) and by Bellar et al. (1974). Narrow-mouth vials with a total volume in excess of 50 ml were considered acceptable (Bellar et al., 1974). It was recommended that the vials be filled to overflowing and each covered with a Teflon-faced silicone rubber septum. Because of the presence of chlorine in some drinking water supplies and the chlorination of organic materials, an increase in the concentration of some halohydrocarbons may occur also during storage (Kopfler et al., 1976).

Bellar et al. (1974) reported that free chlorine can result in the formation of TCI and other halohydrocarbons. In measurements of TCI in water from a sewage-treatment plant, they found an increase of 1.2 µg TCI/l in the water after chlorination. Dietz and Singley (1979) collected water samples in 125-ml serum vials that had been cleaned by detergent wash, distilled water rinse, dichromic acid wash, distilled water rinse, acetone rinse, hexane rinse, acetone rinse, distilled water rinse, and oven-drying at $150^{o}C$. Vials were completely filled and then sealed with Teflon-faced septa and aluminum crimp seals. Samples were stored at $4^{o}C$. Minimum loss occurred during storage at $4^{o}C$ up to 28 days.

The stability of stored samples of TCI present in ambient air or breath samples from exposed individuals has been investigated by Pasquini (1978). The mean percent loss of TCI in alveolar breath samples collected in glass tubes at room temperature was 59.3 ± 15.3 percent over 169 h. The mean decay rate of TCI in dilution air samples was 4.7 ± 3.2 percent over the same time interval. Moisture, temperature, and tube surface and condition can greatly alter vapor retention. For TCI, tubes stored at $37^{o}C$ had high vapor retention rates. When breath was exhaled into the tubes at $37^{o}C$, water vapor was not condensed from the sample. Additional experiments with TCI indicated that siliconized tubes showed a lower decay rate than non-siliconized tubes.

Renberg (1978) judged XAD-4 the most suitable of several possible adsorbents for volatile halogenated hydrocarbons in water, including TCI. This method results in an extract concentrated enough for both chemical determination and small-scale biological tests.

Kummert et al. (1978) have suggested a direct aqueous injection high-pressure liquid chromatography technique for analysis of TCI in natural waters. The technique has been successfully applied to an actual water pollution case for tetrachloroethylene. The detection limit for TCI was reported as 1 μmol l^{-1} for a 10-ml sample.

3.3.3.1 Air-Mixing Ratios--Measurements of ambient air mixing ratios of TCI have been made at a variety of sites in the United States and abroad. As shown in Table 3-7, urban areas have mixing ratios in the low ppb range.

Singh et al. (1979) reported an average mixing ratio in the northern hemisphere of 11 to 17 ppt, with the average in the southern hemisphere of less than 3 ppt. Over the Pacific Northwest, an average tropospheric mixing ratio of 20 ± 3.9 ppt was found (Cronn et al., 1977a). Harsch (1977) reported levels between 22 ppb and 8.7 ppb in more than 20 different indoor environments. Brodzinsky and Singh (1982), in their review of monitoring data, reported that background concentrations average about 30 ppt. A maximum level of 87 ppb was reported for Newark, New Jersey.

3.3.3.2 Water--Trichloroethylene has been detected in a number of raw water sources, finished drinking waters, and subsurface waters in the United States. The occurrence of TCI in waters of the United States has been recently reviewed by the U.S. Environmental Protection Agency (1979).

(1) Drinking water

Dowty et al. (1975) detected TCI in finished drinking water in the New Orleans area. It was reported to pass through the water treatment plant without alteration. It was also reported to be incompletely removed after passage of water through a charcoal filtering unit. Detection was made by GC-MS.

The most comprehensive surface water monitoring effort for TCI to date (Ewing et al., 1977) has shown TCI to be a common low-level (ppb) contaminant of surface waters in the vicinity of urban/industrial areas. TCI was reported at concentrations of 1 ug/l (ppb) or greater in 72 of 179 surface water samples from U.S. urban/industrial areas from 23 states (Table 3-8). Ewing also reported samples of drinking water which were not included in this total.

TABLE 3-7. AMBIENT AIR-MIXING RATIOS OF TRICHLOROETHYLENE

Location	Date of measurements	Mixing ratio (ppb) Maximum	 Minimum	 Average	Reference
ARIZONA					
Phoenix	04/23-05/06 1979	3.0696	0.0117	0.4835 ± .5869	Singh et al., 1981
Grand Canyon*	11/28-12/05 1977	0	0	0	Pellizzari, 1979a
ARKANSAS					
Helena	11/30 1976	≤ 0.03	≤ 0.03	≤ 0.03	Battelle, 1977
El Dorado*	06/13-07/08 1978	13	0.13	3.1 ± 3.8	Pellizzari, 1979a
CALIFORNIA					
Stanford Hills	11/23-11/30 1975	3.09	0.006	0.093 ± 0.32	Singh et al., 1979a
Los Angeles	04/28-05/04 1976	1.77	0.026	0.315 ± .310	Ibid.
Los Angeles	04/09-04/21 1979	1.702	0.363	0.399 ± .302	Singh et al., 1981
Point Reyes	12/01-12/12 1975	0.064	0	0.024 ± 0.014	Singh et al., 1979
Palm Springs	05/05-05/11 1976	0.446	0.006	0.035 ± 0.050	Ibid.
Yosemite	05/12-05/18	0.022	0.008	0.015 ± 0.003	Ibid.
Mill Valley	01/11-01-27 1977	0.090	0.009	0.026 ± 0.094	Ibid.
Riverside	04/25-05/04 1977	0.476	0.057	0.266 ± 0.094	Ibid.
Menlo Park					Singh et al., 1979
Upland	08/13-09/23 1977	0.64	0	0.080 ± 0.22	Pellizzari, 1979a
Badger Pass	05/05-05/13 1977	0.016	0.001	0.012 ± 0.002	Ibid.

TABLE 3-7. (continued)

Location	Date of measurements	Mixing ratio (ppb) Maximum	Minimum	Average	Reference
Point Arena	05/23-05/30 1977	0.012	0.010	0.011 ± 0.001	Ibid.
Point Arena	08/30-09/05 1978	0.034	0.008	0.017 ± 0.007	Ibid.
San Jose	08/21-08/27 1978	2.192	0.048	0.417 ± 0.425	Ibid.
Oakland	06/28-07/10 1979	1.5582	0.0141	0.1876 ± 0.2697	Singh et al., 1981
La Jolla*		3.9	0	1.3 ± 1.2	Su and Goldberg, 1976
DELAWARE					
Delaware City	07/08-07/10 1974	0.56	< 0.05	0.35	Lillian et al., 1975
LOUISIANA					
Baton Rouge*	11/18 1976 05/20 1977	0.65	0	0.4 ± 0.23	Pellizzari et al., 1979; Battelle, 1977
Lake Charles	12/06 1976 06/26 1978	2.1	0.073	1.6 ± 0.79	Battelle, 1977; Pellizzari, 1979b
MARYLAND					
Baltimore	07/11-07/12 1974	< 0.05	< 0.05	-	Pellizzari et al., 1979
NEW JERSEY					
Rutherford*	05/01 1978 12/29 1979	8.6	0	1.15	Bozzelli and Kebbekus, 1983
Somerset *	07/18-07/26 1978	0.28	0	0.17 ± 0.17	Bozzelli and Kebbekus, 1979
South Amboy	01/27 1979 12/29 1979	8.5	0	0.08 ± 0.28 0.4 ± 1.4	Bozzelli and Kebbekus, 1983

TABLE 3-7. (continued)

Location	Date of measurements	Mixing ratio (ppb) Maximum	 Minimum	 Average	Reference
Batso	02/26 1979 12/29 1979	1.3	0	0.08 ± 0.28	Ibid.
Seagirt	06/18-06/19 1974	2.8	< 0.05	0.26	Lillian et al., 1975
Newark	03/23 1976 12/29 1979	1.9	0	0.25	Bozzelli and Kebbekus, 1983
Sandy Hook	07/02-07/05 1974	0.8	< 0.05	0.34	Lillian et al., 1975
Elizabeth	09/15 1978 12/29 1979	6.4	0	0.76	Bozzelli and Kebbekus, 1983
Fords*	03/26 1976 09/27 1978	3.1	0	1.9 ± 1.1	Pellizzari, 1977; Pellizzari et al., 1979
Middlesex*	07/23-07/28 1978	0.36	0	0.22 ± 0.11	Bozzelli and Kebbekus, 1979
Edison*	03/24 1976 09/24 1978	6.1	0.54	2.7 ± 2.1	Pellizzari, 1978; Pellizzari, 1979; Pellizzari, 1977b
Carlstadt*	09/28-09/30 1978	9	3	6.4 ± 3.1	Pellizzari et al., 1979
Camden	04/03-10/24 1979	3.5	0	0.42 ± 0.87	Bozzelli and Kebbekus, 1983
Bayonne	March-Dec. 1973	8.8	< 0.05	0.92	Ibid.
Boundbrook*	03/26 1976 09/20 1978	4.1	0	2.4 ± 1.8	Pellizzari, 1977; Pellizzari et al., 1979
Bridgewater*	07/17 1978 08/05 1978	6.9	0	0.35 ± 0.29	Bozzelli and Kebbekus, 1979
Rahway*	09/20-09/22 1978	18	3.4	11 ± 7.8	Pellizzari et al., 1979

TABLE 3-7. (continued)

Location	Date of measurements	Mixing ratio (ppb) Maximum	Minimum	Average	Reference
NEW YORK					
NYC	06/27-06/28 1974	1.1	0.11	0.71	Lillian et al., 1975
Whiteface Mtns.	09/16-09/19 1974	0.35	< 0.05	0.10	*Ibid.*
Niagara Falls/Buffalo	Fall, 1978	0.11	0.05	0.10	Pellizzari et al., 1979
OHIO					
Wilmington	07/16-07/26 1974	0.63	< 0.05	0.19	*Ibid.*
NEVADA					
Reese River	05/14-05/20 1977	0.016	0.001	0.012 ± 0.002	Singh et al., 1979
KANSAS					
Jetmar	06/01-06/07 1978	0.021	0.007	0.013 ± 0.004	*Ibid.*
WASHINGTON					
Pullman	December 1974-February 1975	-	-	< 0.005	Grimsrud and Rasmussen, 1975
Pacific Northwest	March 1976	-	-	0.020 ± 0.0039	Cronn et al., 1977a
PACIFIC OCEAN					
(lat 37^{o}N)	April 1977	-	-	0.0124	Cronn et al., 1977b
PANAMA CANAL ZONE					
(lat 9^{o}N)	July 1977	-	-	0.015	Cronn and Robinson, 1978

TABLE 3-7. (continued)

Location	Date of measurements	Mixing ratio (ppb)			Reference
		Maximum	Minimum	Average	
NORTH ATLANTIC OCEAN	October 1973	-	-	< 0.005 ± 0.0657	Lovelock, 1974a
WESTERN IRELAND	June-July 1974	-	-	0.015 ± 0.0121	Ibid.
ENGLAND	March 1972	.005	4×10^{-4}	.002	Murray and Riley, 1973a
JAPAN					
Tokyo	09/10-10/27 1975	47.1	0.8	3.5 ± 3.7	Tada et al., 1976
Various sites	Summer, 1979	0.038	0.005	0.014 ± 0.010	Makide, et al., 1980
WESTERN EUROPE	1976	8.5	< 0.02		Correia et al., 1977
COLORADO					
Denver*	06/16-06/26 1980	0.41	0.028	0.2 ± 0.11	Singh et al., 1980
MISSOURI					
St. Louis*	05/30 1980 06/08 1980	0.24	0.019	0.11 ± 0.063	Ibid.
TEXAS					
Aldine	06/22 1977 10/20 1977	0.029	0	0.009 ± 0.017	Pellizzari et al., 1979
El Paso	04/05 1978 05/01 1978	0.3	0	0.15 ± 0.11	Pellizzari, 1979a
Freeport	08/09 1976 11/10 1976	0.029	0	0.001 ± 0.004	Battelle, 1977b; Pellizzari et al., 1979
Houston	07/27 1976 05/24/1980	0.33	0	0.12 ± 0.098	Pellizzari et al., 1979; Singh et al., 1980

TABLE 3-7. (continued)

Location	Date of measurements	Mixing ratio (ppb) Maximum	Minimum	Average	Reference
WASHINGTON					
Auburn*	01/10-01/11 1977	0.83	0.19	0.64 ± 0.29	Battelle, 1977b
WEST VIRGINIA*					
Nitro	09/27 1977 11/18 1977	0.067	0	0.032 ± 0.035	Pellizzari, 1978
West Belle	09/27 1977 11/18 1977	0	0	0	Ibid.
VIRGINIA*					
Front Royale	09/29 1977 11/16 1977	0.009	0	0.003 ± 0.004	Ibid.

*Data obtained from summary report of Brodzinsky and Singh, 1982.

TABLE 3-8. MEAN LEVELS OF TCI IN SURFACE WATERS[a]

	Surface water samples			Mean levels (ug/l)	
State	Positive for TCI	Total	Percent positive for TCI	All samples[b,c]	Positive samples only
Alabama	1	7	14	0.5	1.0
Delaware	4	10	40	1.2	2.3
Illinois	17	25	68	0.6	3.2
Indiana	1	4	25		1.0
Kentucky	1	3	33	0.7	1.0
Louisiana	1	7	14	0.6	1.0
Michigan	1	8	13	23.9	188
Minnesota	2	9	22	1.9	7
New Jersey	14	15	93	4.0	4.3
New York	10	20	50	1.6	2.6
Pennsylvania	14	21	67	2.3	3.2
Tennessee	1	6	17	0.9	3.0
Texas	4	8	50	1.2	1.8
West Virginia	1	3	3	0.7	1.0
All Sites	72	179	40	2.7	5.9

[a] Source: Ewing et al. (1977); drinking water samples not reported in these data.

[b] Mean levels were calculated by substituting a value of one-half the reporting limit of 1 ppb (1 ug/l) for samples reported as having non-detectable (i.e., less than 1 ug/l).

[c] Levels of TCI in sample from the following states (number of sites per state in parentheses) were reported as not detectable (i.e., less than 1 ug/l):

California (9)
Iowa (2)
Mississippi (1)
Missouri (3)
North Carolina (1)
Oregon (3)
Washington (2)
Wisconsin (6)

A concentration of 8.5 ppb was found in the drinking water of the town of Grand Island, New York (Coleman et al., 1976) using headspace GC-EC. In 10 lake water samples from this area, an average concentration of 11.9 ppb ± 2.5 percent was found.

Coleman et al. (1976) detected TCI in the drinking water of four cities in 1975. The concentrations were: 0.1 ppb (Cincinnati); 0.3 ppb (Miami); <0.1 ppb (Ottumwa, Iowa); and 0.5 ppb (Philadelphia). No TCI was detected in drinking water samples for Seattle. Identification and quantification were made by GC-MS using the purge/trap technique.

As evidenced by the differences reported in the individual studies above, the frequency of contamination and the amount of TCI found in potable waters depend in part on the source of water being monitored.

The surface water supplies of 133 cities using surface water supplies have been sampled during federal surveys. Thirty-two percent of the finished waters serving these cities were found to contain TCI. The concentration of TCI in these water supplies ranged from 0.06 to 3.2 ug/l. The average concentration in positive water supplies was 0.47 ug/l. Thirty percent of all supplies contained a level of TCI which was below 1 ug/l. The remaining two percent of the supplies contained a concentration between 1 to 4 ug/l.

In 25 cities that draw their water from underground sources, water supplies were sampled for TCI during the NOMS, NORS, SRI and EPA Region V surveys. Thirty-six percent of these finished waters contained detectable concentrations of this chemical. The median level detected was 0.31 ug/l with a range from 0.11 to 53.0 ug/l.

The Community Water Supply Survey examined an additional 330 ground water systems selected randomly from across the United States. Four percent of these systems contained a detectable level of TCI. Seventy percent of these detectable levels of TCI were between 0.5 to 5 ug/l. TCI was found in finished water from small and large systems alike.

In analyses of drinking waters from sites near producer and user facilities and from a background site, Battelle (1977) found the following concentrations in 1977: 19 ppb (Freeport, Texas); 0.4 ppb (Baton Rouge, Louisiana); 0.1 ppb (Lake Charles, Louisiana); 32 ppb (Des Moines, Iowa); and 22 ppb (Helena, Arkansas).

(2) Rivers, treatment plant effluents

Up to 403 ppb TCI was detected by Battelle in surface waters near specific manufacturing facilities (1977). Trichloroethylene was detected in both industrial wastewater and river water receiving the waste (Jungclaus et al., 1978).

Murray and Riley (1973a) detected TCl in surface sea water in the eastern Atlantic ocean.

Bellar et al. (1974) found an increased concentration of TCl after chlorination treatment of the effluent from a sewage-treatment facility. Before chlorination, the concentration detected by GC-MS was 8.6 ppb; after chlorination, the concentration increased to 9.6 ppb.

Case reports describing TCl contamination of well waters and water supplies have been reviewed elsewhere (U.S. EPA, 1979). Concentrations as high as 330 ppb have been reported.

Correia et al. (1977) measured TCl in river, canal, and sea waters that receive effluent from production and user sites. Values ranged from 0.02 to 74 μg/l (ppb w/w), showing the effect of these additions on background levels. Dilution effects and losses to the surrounding air were noted.

3.3.3.3 Soil and Sediment--Battelle (1977) reported the presence of TCl in soil samples in the vicinity of producers and users at levels up to 5.6 ppb. Trichloroethylene was detected in the soil in the Love Canal area in Niagara Falls, New York (U.S. EPA, 1979).

Pearson and McConnell (1975) found up to 9.9 ppb in sediment from Liverpool Bay, England. Murray and Riley (1973b) found TCl in river mud.

In freshwater sediment taken near the Hooker Chemical facility at Taft, Louisiana, up to 300 ppb TCl was detected (Battelle, 1977).

Rogers and McFarlane (1981) reported that aluminum (Al^{+3})-saturated montmorillonite clay sorbed about 17 percent of the TCl applied (100, 500, and 1,000 ppb). The organic carbon content was assumed to be zero. There was no apparent absorption when calcium-saturated montmorillonite clay was used. With two silty clay loams (organic carbon content ≅ 2 percent), less than 6 percent of the TCl available was sorbed. When soil sorption was normalized, based on the soil organic carbon contents, a correlation was found between water solubility, sorption constant, and the partitioning coefficient.

3.4 ECOLOGICAL EFFECTS

Trichloroethylene has been tested for acute toxicity in a limited number of aquatic species. This section presents observed levels reported to result in adverse effects under laboratory conditions. Such parameters of toxicity are not easily extrapolated to environmental situations. Test populations themselves may not be representative of the entire species in which susceptibility of various lifestages to the test substance may vary considerably.

Guidelines for the utilization of these data in the development of criteria levels for TCI in water are discussed elsewhere (U.S. EPA, 1980).

The toxicity of TCI to fish and other aquatic organisms has been gauged principally by flow-through and static testing methods (Committee on Methods for Toxicity with Aquatic Organisms, 1975). The flow-through method exposes the organism(s) continuously to a constant concentration of TCI while oxygen is continuously replenished and waste products are removed. A static test, on the other hand, exposes the organism(s) to the added initial concentration only. Both types of tests are commonly used as initial indications of the potential of substances to cause adverse effects.

Alexander et al. (1978) used both flow-through and static methods to investigate the acute toxicity of several chlorinated solvents, including TCI, to adult fathead minnows (*Pimephales promelas*). Studies were conducted in accordance with test methods described by the U.S. Environmental Protection Agency (1975). The lethal concentration of each solvent tested to produce 50 percent mortality (LC50) was lower in the flow-through than in the static-type experiment. The flow-through method was considered the better of the two for volatile solvents such as TCI. The 96-h LC50 concentration for TCI was 40.7 mg/l in the flow-through test. The 95 percent confidence band was between 31.4 and 71.8 mg/l. Of four solvents tested (perchloroethylene, methylene chloride, methylchloroform, and TCI), TCI had the second highest toxicity (after perchloroethylene).

In acute static experiments with bluegills, TCI was reported to have a 96-h LC50 value of 44.7 mg/l (U.S. EPA, 1978b). With *Daphnia magna* as the test organism in a 48-h static experiment, the LC_{50} for TCI was 85.2 mg/l. In a chronic test with this organism, no adverse effects were found at the highest test concentrations of 10 mg/l.

Investigation of the bioconcentration potential of TCI for bluegill indicated that the bioconcentration factor (BCF) was 17. The half-life of this compound in tissues was less than 1 day (U.S. EPA, 1980; U.S. EPA, 1978b). These data suggested the unlikelihood of residues at exposure concentrations below those directly toxic to aquatic life (U.S. EPA, 1980).

Few data are available relating to effects of TCI on saltwater aquatic life. Borthwick (1977) exposed sheepshead minnows and grass shrimp to 20 and 2 mg/l, respectively, and noted erratic swimming, uncontrolled movement, and loss of equilibrium after several minutes.

3.5 CRITERIA, STANDARDS, AND REGULATIONS

The current occupational standard for TCI established by the Occupational Safety and Health Administration in 1972 (General Industry Standards, OSHA, 1978) is 100 ppm (525 mg/m^3) as a time-weighted average (TWA) over an 8-h period. More recently, the National Institute for Occupational Safety and Health (NIOSH) (Page and Arthur, 1978) has noted that the current standard appears to be inadequate for protection of workers from the carcinogenic potential of TCI. A level of 25 ppm, as a TWA, was the recommended standard proposed by NIOSH, based on engineering control capabilities.

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4. PHARMACOKINETICS AND METABOLISM

4.1 ABSORPTION AND DISTRIBUTION

Trichloroethylene (1,1,2-trichloroethylene or TCI), a chloroalkene, is a highly volatile liquid (94 torr, 30°C) whose vapors exert pharmacologic effects common to general inhalation anesthetics. Although it is no longer widely used as an anesthetic or analgesic agent, the highly effective fat-solvent properties of TCI are the basis of its numerous industrial uses and its use as an extractant in the food processing industry (Chapter 3.1). At work sites where this compound is manufactured or used, the principal mode of entry into the body is inhalation and lung absorption of TCI vapor. Dermal absorption occurs with direct liquid contact in some degreasing applications and consumer uses (e.g., paint stripping). Oral absorption is also an important route of absorption. Although sparingly soluble in water (1.0 mg/l, 25°C), TCI is present in the water supplies of many of our cities and in their atmospheres, and it has been identified in measurable amounts in the food chain. Hence, ordinary sources in the nonindustrial environment include air, food, and drinking water. Indeed, TCI has been detected in notable amounts in the breath of healthy humans and in postmortem human tissue samples (Conkle et al., 1975; McConnell et al., 1975; Barkley et al., 1980).

4.1.1 Dermal Absorption

Skin absorption from TCI vapor exposure is negligible, although direct skin contact with the liquid by immersion of the hands, or partial body immersion, may result in significant absorption. Uptake through the skin is related to the type of skin exposed and the area of skin exposure as well as to the duration of skin exposure. Tsuruta (1978) estimated skin absorption of TCI in mice by *in vivo* and *in vitro* techniques. The percutaneous absorption rate of TCI in mice was reported to be between 59.8 and 92.4 $\mu mol/min/cm^2$ or between 7.82 and 12.1 $\mu g/min/cm^2$. Tsuruta concluded that direct contact with TCI, even if the percutaneous absorption rate of TCI is different between human skin and mouse skin, may have the potential to contribute importantly to the overall exposure to TCI. However, Stewart and Dodd (1964) have experimentally estimated skin absorption of TCI in volunteers by noting its appearance and concentration in lung alveolar air after thumb immersion. The mean peak concentration of TCI in expired air 30 min after immersion was only 0.5 ppm, indicating that the rate of absorption through the skin is very slow, even after allowing for the possibility of lipid

TABLE 4-1. PARTITION COEFFICIENTS OF TCI FOR VARIOUS BODY TISSUES AND COMPONENTS OF RAT AND MAN

	Mean values
Rat	
Blood/air	25.82
Lung/blood	1.03
Heart/blood	1.10
Kidney/blood	1.55
Liver/blood	1.69
Muscle/blood	0.63
Brain/blood	1.29
Testes/blood	0.71
Spleen/blood	1.15
Fat/blood	25.59
Man	
Blood/air	9.92
Fat/air	674.4
Lecithin/air	387.9
Cholesterol/air	52.15
Cholesterol oleate/air	261.93
Triolein/air	848.24

Adapted from Sato et al., 1977.

Conversion factors:

1 ppm vapor in air = 5.38 mg/m^3 at 25°C, 760 torr.

slower absorption of TCI dissolved in corn oil is probably due to the high lipid solubility (and poor aqueous solubility) of this compound which remains as a depot in the oil. Hence, absorption is limited to the rate of absorption of the corn oil itself, or the corn oil effectively acts as a slow-release dosage form.

4.1.3 Pulmonary Absorption

4.1.3.1 Man--Inhaled TCI rapidly equilibrates across the lung alveolar endothelium. The blood/gas partition coefficient of 9.92 at 37°C is comparable to the anesthetic gases--chloroform, diethylether, and methoxyfluorane--but its lipid solubility is considerably higher (olive oil/gas coefficient, 960 at 37°C) (Powell, 1947; Eger and Larson, 1964). Thus, pulmonary uptake of TCI is rapid and extensive with considerable distribution into lipid of body tissues (Table 4-1).

Pulmonary uptake is proportional to the magnitude of the gas partial pressure difference between alveoli and pulmonary venous blood. This difference diminishes when all body tissues achieve equilibrium with the alveolar (arterial) partial pressure. Initially, uptake is rapid, but about 8 h exposure is required for complete tissue equilibrium to be achieved (Fernandez et al., 1977; Sato et al.; 1977; Monster et al., 1976, 1979; Muller et al., 1974). Figure 4-1 shows that equilibrium is achieved most rapidly (4 to 6 h) with the rich blood-perfused tissues. The poorly perfused fat tissues (FG) require longer than 8 h for equilibrium.

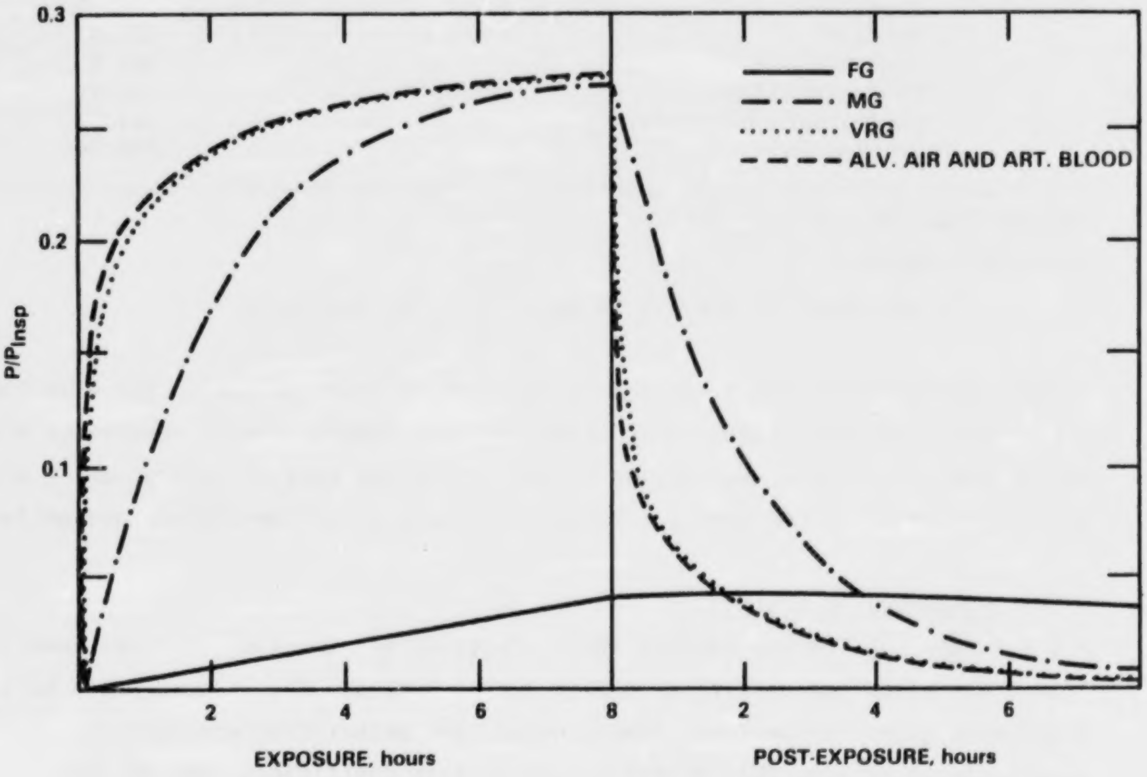


Figure 4-1. Predicted partial pressure of TCI in alveolar air and tissue groups during and after an 8-h exposure of 100 ppm. From Fernandez et al. (1977).

At equilibrium with a given concentration of TCI in inspired air, the difference between the inspired air concentration of TCI and alveolar concentration of TCI provides a measure of gas uptake. With appropriate accounting for ventilation (about 4-8 l/min at rest) and duration of exposure, the amount taken up by the body, or retention (dose; body burden), can be calculated. At alveolar and tissue equilibrium, the retention reflects disposition within the body by tissue storage, metabolism, or excretion by routes other than the lungs. Retention of 36 to 75 percent has been reported for TCI by various investigators (Soucek and Vlachova, 1960; Bartonicek, 1962; Nomiyama and Nomiyama, 1971, 1974a,b), with higher values (70 to 75 percent) reported from more recent studies (Fernandez et al., 1975; Monster et al., 1976, 1979) (Tables 4-2 and 4-3). The retention value is independent of the inspired TCI concentration. In contrast, the amount (dose) of TCI absorbed into the body is directly proportional to the TCI concentration in the inspired air (Fernandez et al., 1975; Monster et al., 1976, 1979; Muller et al., 1974). The body burden of TCI also increases with duration of exposure, repeated daily exposures and exercise (increased ventilation) at a given inhaled air concentration (Astrand and Ovrum, 1976; Monster et al., 1976, 1979). These relationships between inspired air concentration pulmonary uptake and body burden are illustrated by the data of Astrand and Ovrum and of Monster et al. in Tables 4-2 and 4-3. Astrand and Gamberale (1978) exposed volunteers to 100 and 200 ppm (ventilation volume 9.3 l/min) for 70 min and observed a linear relationship between pulmonary uptake (Q) and alveolar concentration (A) divided by inspired air concentration (I)

$$Q = 0.72 \left(\frac{A}{I}\right) + 74.91.$$

However, as about eight or more hours are required for complete body equilibration, this relationship only approximates steady-state inhalation conditions.

The blood concentration of TCI during inhalation and in the elimination phase after exposure closely parallels alveolar gas concentration (Figure 4-1 and Figure 4-2) (Muller et al., 1974; Monster et al., 1976, 1979; Stewart et al., 1974; Sato et al., 1977; Fernandez et al., 1977). The kinetics of tissue uptake, accumulation, and equilibration of TCI is a function of blood concentration, the blood flow/tissue mass, and the relative solubilities of TCI in

TABLE 4-2. PULMONARY UPTAKE OF TCI FOR 25 VOLUNTEERS EXPOSED TO TCI FOR FOUR CONSECUTIVE 30-MIN PERIODS AT REST AND DURING EXERCISE (50-150 WATTS). FROM ASTRAND AND OVRUM (1976).

Exposure concentration (mg/m^3)	Conditions	Amount inhaled mg	Amount taken up* mg
540	rest	149 ± 6	79 ± 4
1080	rest	313 ± 15	156 ± 9
540	Exercise (50w)	395 ± 6	160 ± 5
1080	Exercise (50w)	803 ± 12	308 ± 16

540 and 1080 mg/m^3 ≡ 100 and 200 ppm respectively.

*Calculated from difference in inspired air and expired air x ventilation volume x time. Retention value averaged = 45 percent.

TABLE 4-3. PULMONARY UPTAKE OF TCI FOR 4 SUBJECTS AT REST EXPOSED TO 70 AND 140 PPM TCI FOR 4-HOUR PERIODS AND 3-HOUR PERIODS, PLUS TWO 30-MIN PERIODS OF WORK (100 WATTS). FROM MONSTER (1976).

Exposure concentration and conditions	Dose (mg) Subject				Mean mg
	A	B	C	D	
70 ppm, rest	320	430	330	470	390
70 ppm, rest + work	500	450	470	660	520
140 ppm, rest	740	790	710	790	755
140 ppm, rest + work	1050	1100	970	1100	1055

Retention value average ≡ 38 percent.

tissues (Table 4-1). Therefore, TCI most rapidly attains equilibrium by passive diffusion into the vessel-rich group (VRG) of tissues (brain, heart, kidneys, liver, and endocrine and digestive systems), more slowly with the lean mass (MG) (muscle and skin), and last with adipose tissue, i.e., fat groups (FG). As determined from elimination kinetics following exposure, TCI distributes from blood into these three major compartments at approximate rate constants of 17 hr^{-1} ($t_{1/2}$, 2.4 min) for VRG, 1.7 hr^{-1} ($t_{1/2}$, 25 min) for MG, and 0.2 hr^{-1} ($t_{1/2}$, 3.4 hr) for FG (Fernandez et al., 1975, 1977; Sato et al., 1977). Though the lean muscle mass is 50 percent of the body volume and adipose tissue is 20 percent, saturation and desaturation proceed more rapidly from the MG compartment ($t_{1/2}$, 25 min) than from the FG compartment ($t_{1/2}$, 3.4 hr) because of the considerably greater solubility of TCI in lipid (Table 4-1). Interperson variations in TCI uptake (dose) are therefore influenced primarily by lean body mass and secondarily by adipose tissue mass (Monster et al., 1976, 1979).

4.1.3.2 Animals--Stott et al. (1982) have determined the pulmonary uptake of TCI in mice and rats exposed to 10 and 600 ppm (54 and 3228 mg/m^3). The animals were exposed in a 30-l glass inhalation chamber to ^{14}C-TCI, the concentration of which was maintained within 10 percent of target. At termination of exposure (6 h), the cumulative pulmonary uptake was estimated by determining the radioactivity

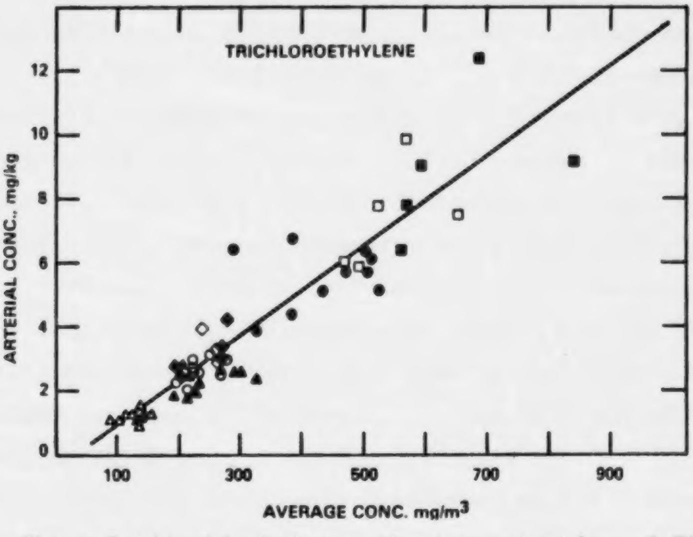


Figure 4-2. The relationship between the concentration of TCI in arterial blood and alveolar air at the end of inhalation exposures (30-min periods, for 15 male subjects). Symbols stand for one exposure period for a subject at rest, and at various levels of exercise. The regression line is $y = -0.489 + 0.014 X$. From Astrand and Ovrum (1976).

of carcass, and of TCI and metabolites in expired air, urine and feces. The data for mice and rats are given in Tables 4-4 and 4-5, respectively. For mice, the body burden for 6-h exposure to 10 and 600 ppm (54 and 3228 mg/m^3) were 78.5 and 3138 μmol equivalents/kg (103.0 and 412.3 mg/g) respectively. For rats, amounts were 35.8 and 1075 μmol equivalents/ kg (4.7 and 141.3 mg/kg) respectively. For these species the 6-h pulmonary uptake was not directly proportional to inspired air concentration level, as observed in man. Furthermore, the resultant body burden to a given TCI-inspired concentration on a kilogram body weight basis is highest for mouse (69 mg/kg/h/600 ppm), intermediate for rat (24 mg/kg/h/600 ppm) and lowest for man (12 mg/kg/h/600 ppm). Presumably, these differences are primarily related to the differences of metabolic and ventilation rates, and to metabolism of TCI by these species. Stott et al. (1982) estimate that man metabolizes 20 times less TCI on a weight basis than rats.

4.1.4 Tissue Distribution and Concentrations

TCI distributes to all body tissues and appears in sweat and saliva (Bartonicek, 1962). It crosses the blood-brain barrier (anesthetic effect) and the placental barrier. Helliwell and Hutton (1950), investigating the distribution in pregnant sheep and goats, found that TCI appeared in the fetal circulation within 2 min of exposure; at equilibrium, the fetal/maternal blood concentration ratio was unity. The partition coefficients for various tissues of rat and man are given in Table 4-1 (from Sato et al., 1977).

4.1.4.1 Man--There is little direct analytical information of tissue levels after various exposure concentrations of TCI. Tissue concentrations are expected to be proportional to exposure concentrations and to duration of exposure. Fernandez et al. (1977), from their mathematical model of TCI uptake, distribution, and excretion, based on experimental data, have predicted the partial pressure of TCI in the three major compartmental tissue groups, vessel-rich group (VRG), muscle group (MG), and adipose tissue (FG), during and after an 8-h inhalation exposure of 100 ppm (538 mg/m^3). Figure 4-1 shows that VRG and MG are nearly always in equilibrium with arterial blood concentration, but the concentration of TCI in FG increases only very slowly and continues to increase beyond the 8-h exposure. During desaturation, TCI in FG exhibits a long residence (about 40 h) for disappearance with detectable FG concentrations remaining even after 70 h. Because of the long residence of TCI in FG, repeated daily exposure

TABLE 4-4. RECOVERY OF RADIOACTIVITY FOR 50-HOUR POSTINHALATION EXPOSURE OF MALE B6C3F1 MICE TO 10 OR 600 PPM ^{14}C-TCI FOR 6 HOUR[1] (From Stott et al., 1982).

	10 ppm			600 ppm		
	µmol-eq TCI/ kg body weight		%[2]	µmol-eq TCI/ kg body weight		%[2]
Expired						
1,1,2-TCI	0.617	(0.182)	0.79	76.2	(27.3)	2.4
CO$_2$	7.38	(0.383)	9.4	299	(37.5)	9.5
Urine	58.1	(18.8)	74.0	2278	(505.0)	72.6
Feces	2.92	(0.542)	3.7	115	(24.6)	3.7
Cage wash[3]	1.10	(0.446)	1.4	85.5	(65.5)	2.7
Skin	2.33	(1.13)	3.0	56.3	(11.9)	1.8
Liver	2.40	(0.401)	3.1	72.2	(10.4)	2.3
Kidney	0.310	(0.310)	0.39	11.1	(3.18)	0.35
Carcass	1.50	(0.316)	1.9	146	(57.9)	4.6
Total body burden	78.5	(17.9)		3138	(616.0)	
Total metabolized	77.9	(18.1)	99.2	3062	(592.0)	97.6

[1] Average of four animals (±SD)

[2] Percentage of recovered radioactivity.

[3] Primarily due to urine.

TABLE 4-5. RECOVERY OF RADIOACTIVITY FOR 50-HOUR POSTINHALATION EXPOSURE OF MALE OSBORNE-MENDEL RATS TO 10 OR 600 PPM ^{14}C-TCI FOR 6 HOUR[1] (From Stott et al., 1982).

	10 ppm			600 ppm		
	µmol-eq TCI/ kg body weight		%[2]	µmol-eq TCI/ kg body weight		%[2]
Expired						
1,1,2-TCI	0.778	(0.103)	2.1	227	(6.40)	21.1
CO$_2$	1.71	(0.295)	4.8	31.2	(2.10)	2.9
Urine	22.6	(5.96)	63.1	594	(79.8)	55.3
Feces	2.52	(0.253)	7.0	38.3	(3.47)	3.6
Cage wash[3]	0.357	(0.204)	1.0	15.1	(10.7)	1.4
Skin	3.78	(1.21)	10.6	76.2	(30.3)	7.1
Liver	1.01	(0.085)	2.8	16.3	(1.13)	1.6
Kidney	0.131	(0.009)	0.37	1.72	(0.083)	0.16
Carcass	2.82	(0.898)	7.9	75.1	(27.7)	7.0
Total body burden	35.8	(5.64)		1075	(87.2)	
Total metabolized	35.0	(5.64)	97.8	847	(81.6)	78.9

[1] Average of four animals (±SD)

[2] Percentage of recovered radioactivity.

[3] Primarily due to urine.

(6 h/day; 100 ppm) results in an accumulated concentration, as TCI from new exposures adds to residual concentration from previous exposures, until steady-state is reached. This accumulation of TCI in FG is shown in Figure 4-3. Adipose tissue levels of TCI reach a steady-state "plateau" (dependent on the inhalation concentration and daily period of exposure) and thereafter remain relatively constant. For a 6-h per day, 100 ppm (538 mg/m^3)-exposure, Fernandez et al. (1977) predict that FG peak plateau concentration occurs 5 to 7 days after initial daily exposure.

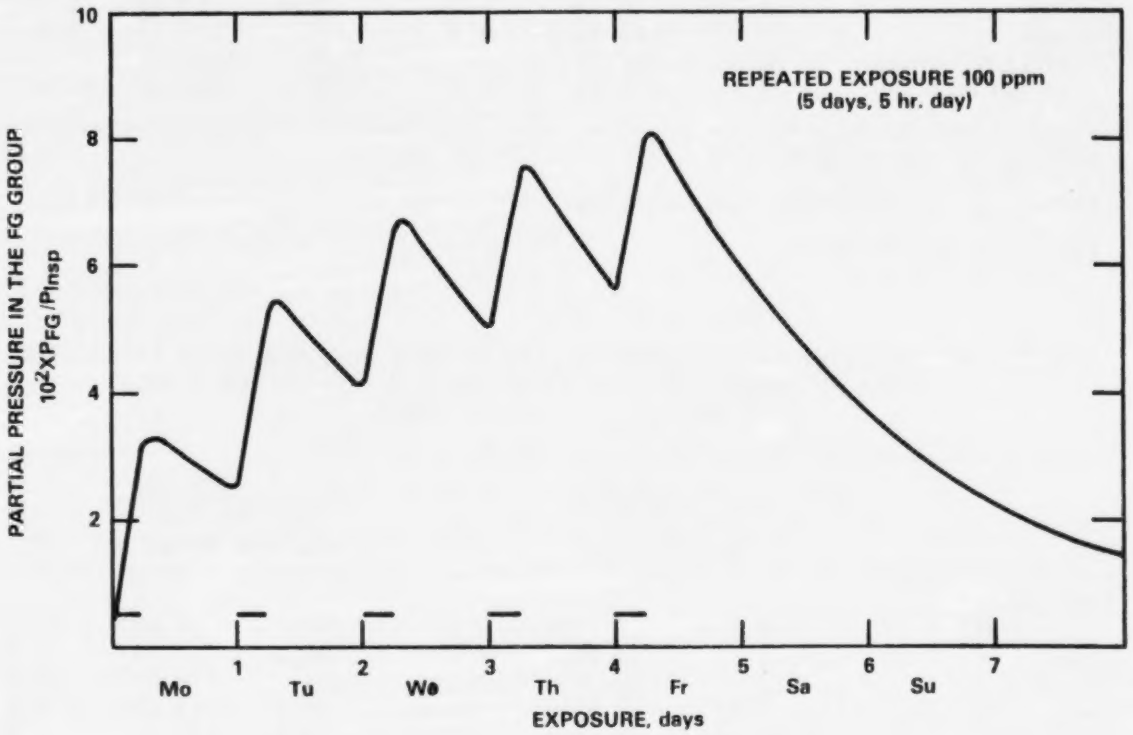


Figure 4-3. Predicted partial pressure of TCI in fatty tissue for a repeated exposure to 100 ppm, 5 days, 6 h per day. From Fernandez et al. (1977).

4.1.4.2 Rodents--Following intravenous administration of TCI to Wistar rats, Withey and Collins (1980) found a whole-body half-time of disappearance from the rat of 2 to 6 min (dependent on dose) but a disappearance half-time of 215 min for adipose tissue, indicative of the propensity of TCI to remain in adipose tissue as compared to other tissues. Pfaffenberger et al. (1980), after dosing rats by gavage for 31 days (1 and 10 mg/d), found that blood serum levels of TCI were not detectable (analyzed 2 h after last dosing); but adipose tissue levels averaged 0.28 and 20.0 μg TCI/g, respectively. Three days after last dosing, 1 μg TCI/g fat still remained in adipose tissues.

Savolainen et al. (1977) analyzed tissue levels of TCI after exposure of rats 6 h per day for 5 days to 200 ppm (1076 mg/m^3) TCI. Table 4-6 gives the values found. Seventeen hours after exposure on day four, blood and adipose tissue levels were 0.35 and 0.23 moles/g, respectively, indicating long storage in adipose tissue, though other tissues contained virtually no TCI. With exposure on day five, tissue levels in brain, lungs, liver, fat and blood reached a steady-state within 2 to 3 h. Partition coefficients were adipose tissue/blood, 10.4; brain/blood, 1.3; lungs/blood, 0.8; and liver/blood, 0.5. The lower value for liver possibly reflects metabolism occurring in this organ, while the high value for adipose tissue reflects high solubility of TCI in lipids.

4.2 EXCRETION

The total elimination of absorbed TCI involves two major processes, pulmonary excretion of unchanged TCI and hepatic biotransformation to urinary metabolites. The details of hepatic biotransformation of TCI are reviewed below. In man, the principal metabolites are trichloroethanol (TCE), TCE-glucuronide, and trichloroacetic acid (TCA). These metabolites are excreted in urine, and in less significant quantities by other excretion routes, such as the bile (Bartonicek, 1962).

4.2.1 Pulmonary Elimination in Man--Pulmonary elimination of unchanged TCI after exposure occurs nearly as an inverted reproduction of its uptake. Alveolar concentration parallels blood concentration; both follow exponential concentration decay curves showing three major components of approximate half-times of 2 to 3 min, 30 min, and 3.5 to 5 h (Figures 4-1 and 4-4), usually represented by VRG, MG, and FG compartments, respectively (Fernandez et al., 1977; Sato et al., 1977; Muller et al., 1974; Nomiyama and Nomiyama, 1971; Monster et al., 1979).

TABLE 4-6. ORGAN CONTENTS OF TCI AFTER DAILY INHALATION EXPOSURE OF 200 PPM FOR 6 HOUR PER DAY. MEASUREMENTS MADE ON FIFTH DAY. From Savolainen et al. (1977).

Time (h of exposure) on fifth day	Cerebellum	Cerebrum	Lungs	Liver	Perirenal fat	Blood
				nmol/g		
0 [17 h after day 4-exposure]	0	0	0.08	0.04	0.23 ± 0.09	0.35 ± 0.1
2	11.7 ± 4.2	9.9 ± 2.7	4.9 ± 0.3	3.6	65.9 ± 1.2	7.5 ± 1.6
3	8.8 ± 2.1	7.3 ± 2.2	5.5 ± 1.4	5.5 ± 1.7	69.3 ± 3.3	6.6 ± 0.2
4	7.6 ± 0.5	7.2 ±1.7	5.8 ± 1.1	2.5 ± 1.4	69.5 ± 6.3	6.0 ± 0.9
6	9.5 ± 2.5	7.4 ± 2.1	5.6 ± 0.5	2.4 ± 0.2	75.4 ± 14.9	6.8 ± 1.2

Values are mean of 2 determinations ± range.

Sato et al. (1977) developed a physiological kinetic model for TCI excretion in man, based on experimental findings from controlled exposures of volunteers. Four male medical students (av. b.w. 61.6 kg) inhaled 100 ppm (538 mg/m^3) for 4 h. After cessation of exposure, the blood and exhaled air concentrations of TCI and the concentration of urinary metabolites were determined during the time-course of 10-h postexposure. Figure 4-4 shows the results obtained. The time-course of concentration decay (C) TCI in blood and exhaled air is the sum of three exponentials:

$$C_{air} = 0.213E^{-23.6t} + 0.029E^{-1.48t} + 0.0126E^{-0.18t}$$

$$C_{blood} = 0.115E^{-16.7t} + 0.449E^{-1.71t} + 0.255E^{-0.20t}$$

These reflect first-order excretion from 3 compartments (VRG, MG, and FG, respectively) of the model (Figure 4-4). As indicated in the model, C_{air} represents pulmonary clearance only, but C_{blood} represents whole-body clearance by

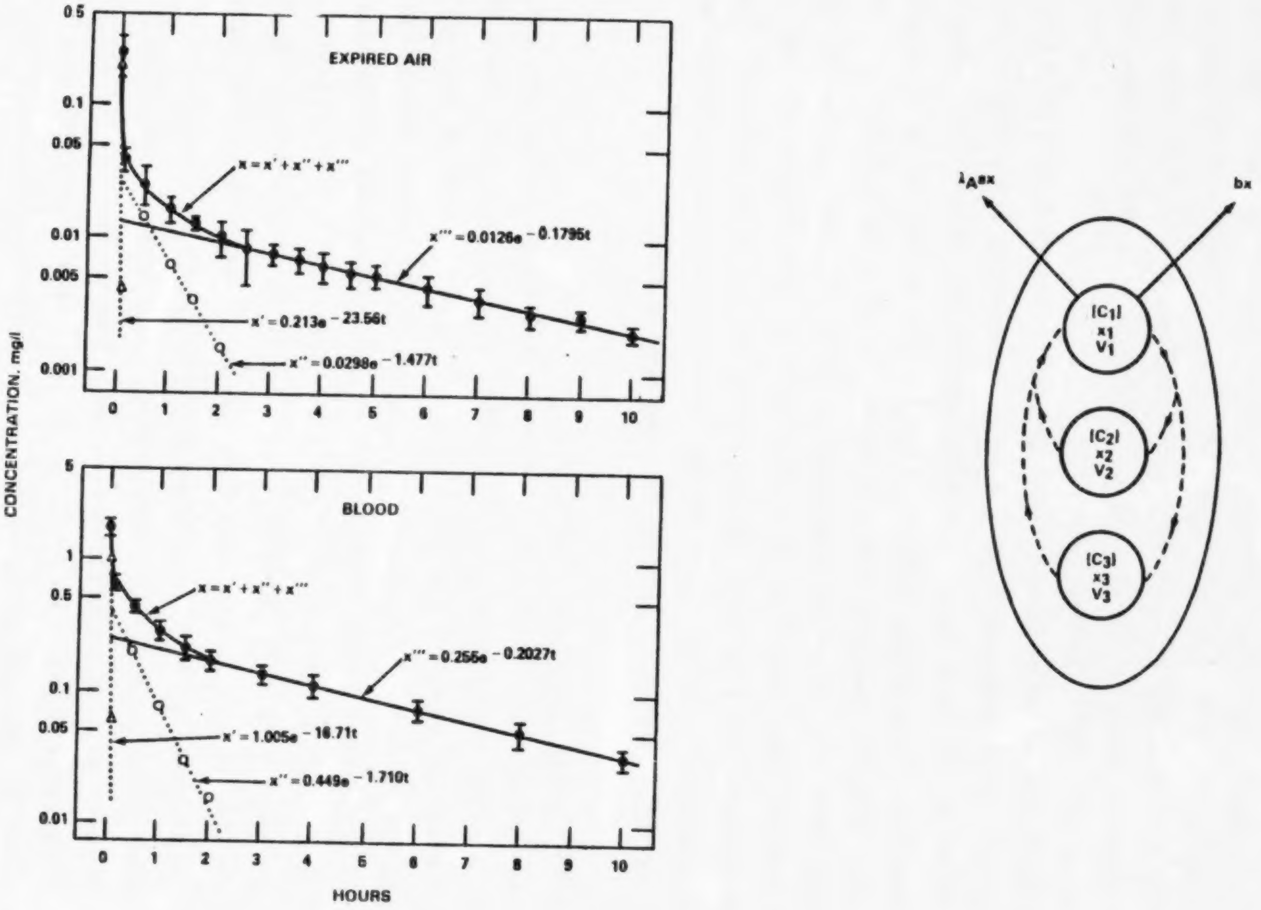


Figure 4-4. Elimination curves of TCI and kinetic model for transfer of TCI in the body. $\lambda\ \alpha\ \chi$ = pulmonary excretion (ventilation x air/blood partition coefficient x blood concentration); bx = metabolic clearance (urine) x blood concentration; v_1, V_2, V_3 volumes of VRG, MG, and FG compartments, respectively. From Sato et al. (1977).

the pulmonary system and metabolism. The kinetic parameters were determined as follows: V (volume of FRG, MG, and FG compartments), 8, 31 and 10/l, respectively; metabolic clearance, 104 l/hr; partition coefficients (blood/air, VRG/ blood, MG/blood, FG/blood), 10, 1.5, 1.0, 67; $t_{\frac{1}{2}}$ of FG, 3.4 h. Similar tri-exponential curves for decay of blood and exhaled air concentrations of TCI have been reported by other investigators (Stewart et al., 1970, 1974; Nomiyama and Nomiyama, 1974; Fernandez et al., 1975, 1977; Monster, 1976).

The long half-time of elimination from adipose tissue (3.5 to 5 h) explains why, after a single short exposure (e.g., 4 to 6 h), exhaled air contains a notable concentration of TCI 18 h later, and also why accumulation of TCI (body burden) occurs with repeated, fluctuating daily exposures until steady-state is reached (Section 4.1.4). Several investigators (Fernandez et al., 1977; Monster et al., 1979) have shown that, for volunteers exposed to 70 to 100 ppm (377 to 538 mg/m^3) 4 h daily for 5 days, the concentration of TCI in blood and exhaled air was two to three times higher 18 h after the last exposure than at the comparable time after a single exposure. TCI accumulation was highest in those volunteers with a proportionally larger ratio of adipose to lean muscle mass.

4.2.2 Urinary Metabolite Excretion in Man

The urinary metabolites of TCI (total amount, excretion ratios, and excretion time-course) have always been of considerable interest over the years as quantitative indices of exposure and body burden. Recent investigations have determined that the half-time for renal elimination of TCE and of TCE-glucuronide approximates 10 h (range 7 to 14 h) after TCI exposure (Nomiyama and Nomiyama, 1971; Muller et al., 1974; Sato et al., 1977; Monster et al., 1976, 1979). This value is in agreement with that observed after ingestion of TCE itself (Breimer et al., 1974; Muller et al., 1974). As might be predicted from this relatively short half-time of renal elimination, blood concentrations of TCE and TCE-glucuronide after repeated daily TCI exposures reach a "plateau concentration" (with peaks for each daily exposure) that is dependent on the inhaled air concentration of TCI (Monster et al., 1979; Muller et al., 1974; Fernandez et al., 1977). Consequently, the same daily amount of TCE and TCE-glucuronide is excreted into the urine during repeated daily exposures to the same air concentration of TCI.

In contrast to the short half-life of TCE, the half-time of renal elimination of TCA produced during TCI exposure approximates 52 h (range 35 to 70 h)

(Soucek and Vlachova, 1960; Nomiyama and Nomiyama, 1971; Muller et al., 1974; Sato et al., 1977; Monster et al., 1976). Similar values have been observed when TCA is directly administered to men (82 h, Paykoc and Powell, 1945; 51 h, Muller et al., 1974). Distribution of TCA in the body is restricted to the extracellular water space (Paykoc and Powell, 1945); it is very tightly and extensively bound to plasma protein and excluded from erythrocytes, so that plasma concentration is twice as high as whole blood (Soucek and Vlachova, 1960; Monster et al., 1979). As a consequence of the long half-life of TCA, and of the constant metabolic formation of TCA from TCE, blood concentrations of TCA smoothly rise during a single TCI exposure, reaching a maximum 24 to 48 h post-exposure before declining exponentially for 10 to 15 days. The blood concentration of TCA during the rising phase and at maximum is proportional to the TCI inspired air concentration and the duration of the exposure, i.e., to the retained TCI dose (Soucek and Vlachova, 1960; Monster et al., 1976; Sato et al., 1977; Fernandez et al., 1977). During repeated daily exposures, TCA accumulates in the blood exponentially, and declines only 24 to 48 h after the last exposure (Muller et al., 1974; Fernandez et al., 1977; Monster et al., 1979). Consequently the daily amount of TCA excreted into the urine increases during repeated exposure and then decreases slowly over a period of 15 to 20 days. Several investigators have documented an apparent increase in the half-time of renal elimination of TCA (to 100+ h) with repeated daily exposures to TCI. This phenomenon is explained by continuous formation of TCA from accumulated tissue stores of TCI and TCE, and by the very tight binding of TCA to plasma protein.

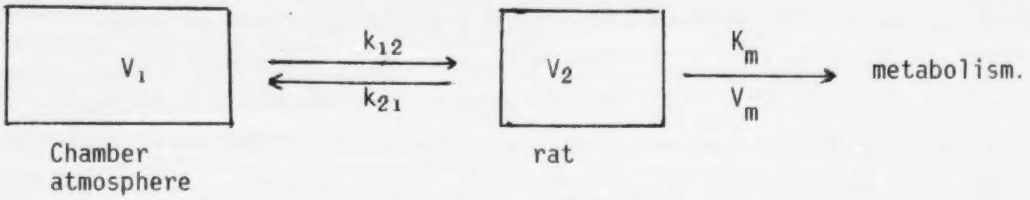
The renal elimination kinetics of TCE and TCA readily explain the urinary excretion ratio time-course of these metabolites. The ratio of daily urinary metabolites, TCE/TCA (mg/day), is highest (2:1) 24 h after a single TCI exposure or after repeated daily exposures (Soucek and Vlachnova, 1960; Muller et al., 1972, 1974; Fernandez et al., 1975; Nomiyama and Nomiyama, 1971, 1977; Sato et al., 1977; Monster et al., 1976, 1979). Thereafter, the relationship of TCE/TCA progressively decreases from day to day and becomes less than unity at about the sixth day following exposure. Several additional factors are known to influence the excretion ratio, TCE/TCA. The ratio is reported to be markedly greater in males than females for the first 24 h after exposure (Nomiyama and Nomiyama, 1971, 1977), and TCA urinary excretion is reported to vary diurnally (Soucek and Vlachova, 1960; Monster et al., 1979). There is no evidence in man that the elimination of TCI by metabolism is saturable, at least up to 300 ppm (1584 mg/m^3) inhalation concentrations, but saturation may occur at higher levels (Section 4.4.2).

4.2.3 Excretion Kinetics in the Rodent

Withey and Collins (1980) determined the kinetics of distribution and elimination of TCI from blood of Wistar rats after intravenous administation of 3, 6, 9, 12, or 15 mg/kg of TCI given in 1 ml water interjugularly. For the three lower doses, the blood decay curves exhibited two components of exponential disappearance and best fitted a first-order two-compartment model, with average kinetic parameters of K_e, 0.18 min^{-1}; V_d, 158 ml; k_{12} and k_{21}, 0.059 and 0.39, respectively. For the highest dose (15 mg/kg), these investigators found that their data best fitted a first-order three-compartment model. They suggest that the shift from two- to three-compartment kinetics may have occurred either because of a dose-related alteration in the kinetic mechanisms of uptake, distribution, metabolism, and elimination, or that three-compartment kinetics were followed at all dose levels, but with lower doses, the third exponential component, representing the third compartment, was obscured by the limit of analytical sensitivity. The kinetic values for the three-compartment model (using a dose of 15 mg/kg) were k_e, 0.174 min^{-1}; V_d, 111 ml; k_{12}, k_{21}, k_{13}: 0.183, 0.164, and 0.051, respectively. The half-time, $t_{½}$, for disappearance of TCI from perirenal fat was 217 min. Withey and Collins (1980) state that they found no evidence in their kinetic analysis of the disposition of intravenous bolus injections of TCI into the rat of nonlinear or dose-dependent Michaelis-Menten kinetics, although the first-order kinetic parameters obtained were clearly dose-dependent. These workers suggested that a dose of 15 mg/kg TCI to the rat is below hepatic metabolism saturation.

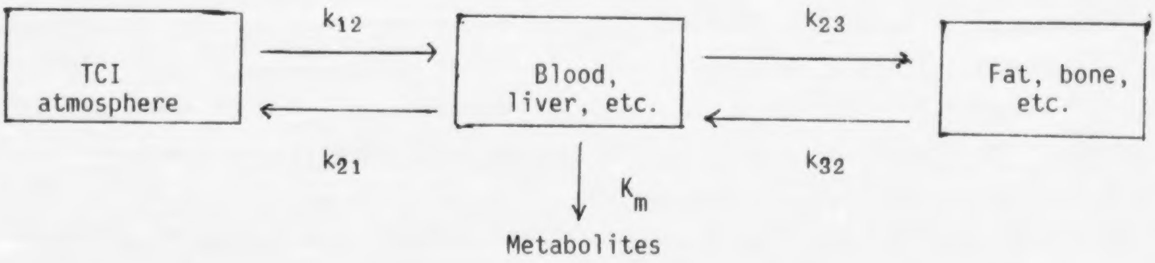
Other investigators have found evidence of dose-dependent Michaelis-Menten kinetics in rats. Stott et al. (1982) exposed rats and mice to 10 and 600 ppm (54 and 3228 mg/m^3) TCI for 6 h. For exposed mice, the body burden (dose) from the 6-h exposure was estimated as 78.5 µmoles of TCI/kg body weight (10 mg/kg) and 3138 µmoles of TCI/kg (412 mg/kg), respectively. These inhalation doses were virtually completely metabolized (Table 4-4). In contrast, for rats (Osborne-Mendel), the inhalation doses were 35.8 µmoles/kg (4.7 mg/kg) and 1075 µmoles/kg (141 mg/kg), respectively, of which 98 percent at the lower dose was metabolized, but only 79 percent at 600 ppm (3228 mg/m^3) (Table 4-5). These results indicate a saturation of metabolism in the rat. Support for this conclusion is found also in the observation that pulmonary elimination of unchanged TCI was only 2 percent of the dose from 10 ppm (54 mg/m^3) exposure but 21 percent of the dose at 600 ppm (3228 mg/m^3) (Table 4-5).

Filser and Bolt (1979) exposed rats (Wistar) to TCI in a closed system (desiccator jar chamber) and determined, from disappearance of TCI from the chamber the inhalation pharmacokinetics, in accordance with the following model:



They found that the metabolism of TCI was dose-dependent in the rat, with the saturation point occurring at 65 ppm (350 mg/m^3). Zero order V_{max} was 210 μmol/h/kg and first-order (below 65 ppm) clearance was 77 l/hr/kg b.w.

Andersén et al. (1980) also analyzed the pharmacokinetics behavior of TCI in rats (Fisher 344) using a Michaelis-Menten approach to demonstrate dose-dependent metabolism. Exposures were conducted in a 31-l battery jar chamber, and the rate of depletion of atmosphere TCI was determined for 30, 100, 1000, 3500, and 8000 ppm (161, 538, 5380, 18830, and 43040 mg/m^3). TCI uptake was fitted to a four-compartment model as follows:



The metabolism of TCI was found to be dose-dependent, with a saturation point of about 1000 ppm (5380 mg/m^3) (K_m, 463 ppm). The kinetic parameters, calculated as molar equivalents TCI, were V_{max}, 185 μmoles/kg/h; K_m, 292 μmoles/l plasma; and first-order rate constant of 0.41 μmoles/h/kg for uptake from the 31-l chamber A "whole animal"/air partition coefficient of 15.4 (as compared to q for blood/air) was calculated and suggested to be a more accurate estimate of microsomal-to-gas distribution than a blood/gas constant.

4.3 MEASURES OF EXPOSURE AND BODY BURDEN

Trichloroethylene is principally absorbed by the lungs during industrial or ambient-air exposure. However, as worker exposure periods and air concentrations are likely to vary, air analysis does not necessarily provide a reliable indication of true exposure. An index of a worker's current exposure and total body burden can be estimated in accordance with the amount of TCI absorbed and retained as predicted by the known pharmacokinetics and metabolism of TCI. Therefore, monitoring TCI levels in blood or expired air and measuring urinary metabolites are the most common methods for determining the body burden (Stewart et al., 1962, 1970, 1974; Morgan et al., 1970; Nomiyama, 1971; Ogata et al., 1971; Ertle et al., 1972; Ikeda et al., 1972; Muller et al., 1972, 1974; Pfäffli and Blackman, 1972; Kimmerle and Eben, 1973a,b; Lowry et al., 1974; Vesterberg et al., 1976; Fernandez et al., 1975, 1977; Ikeda, 1977; Monster et al., 1976, 1979). These methods, however, are subject to high interpersonal variations in the pharmacokinetics and metabolism of TCI that limit their predictive value. Some of the factors now known to contribute to these variabilities are pulmonary dysfunctional states, interindividual differences in intrinsic liver metabolism capacity for TCI, modification of metabolism by drugs and environmental xenobiotics, age, sex, exercise or work load, and body anthropometry. Stewart and his associates (1974) have been vigorous proponents of the value of postexposure alveolar concentrations of TCI as measures of recent exposure and body burden. However, this method is highly dependent on time of sampling and influenced by individual variations in metabolism of TCI. Muller et al. (1974) have concluded that neither TCA excretion nor the sum of urinary metabolites can be taken as representative parameters of previous exposure, except possibly for 24-h daily collections. These workers favor either alveolar TCI concentrations or blood analysis of TCA or TCI as the best present indicators of exposure and body burden. Monster et al. (1979) concluded from their studies that the most "promising parameter for biological monitoring in repeated exposure to TCI" is blood TCA concentration level, because of the ease of sampling and GC analysis, the greater independence of time after exposure, accumulation with chronic exposure and increased body burden, and the smaller interindividual variation of this parameter.

Greater and more precise information than is now available on the absorption, distribution and metabolism kinetics of TCI is required for better means of estimating or predicting body burdens with exposure. To this end, many investigators have developed mathematical models formulating the pharmacokinetics of inhaled TCI and its metabolism (Fernandez et al., 1977; Droz and Fernandez, 1978; Feingold and Holaday, 1977; Gobbato and Mangivachhi, 1979; Sato et al., 1977; Sato, 1979).

4.4 METABOLISM

4.4.1 Known Metabolites

It has been known for 50 years that TCI is extensively metabolized in the body to trichloroethanol (TCE), TCE-glucuronide, and trichloroacetic acid (TCA). Following the introduction in the 1930's of TCI as a general anesthetic, Barrett and co-workers (1939) isolated TCA from the urine of dogs and human patients anesthetized with TCI. Powell (1945, 1947) demonstrated the presence of TCA in both plasma and urine. She also observed that TCA continued to be excreted for more than 2 days after a single anesthetic exposure. Butler (1949), who had previously discovered that the hypnotic, chloral hydrate, was metabolized to TCE, TCE-glucuronide, and TCA (Butler, 1948), hypothesized that this widely used hypnotic was an intermediate metabolite of TCI. His experiments with dogs exposed to TCI clearly demonstrated that the principal metabolites of TCI and TCE were TCE-glucuronide ("urochloralic acid") and TCA (Figure 4-1). However, Butler could not demonstrate the presence of chloral hydrate in either plasma or urine. Nonetheless, this demonstratation of biotransformation of TCI to TCE as well as to TCA was the first proof that TCI anesthetic was converted to a metabolite with hypnotic properties. Trichloroethanol has a hypnotic potency comparable to that of chloral hydrate (Marshall and Owens, 1954). Since that time, the intermediate metabolite, chloral hydrate, has been found in the plasma of rats (Kimmerle and Eben, 1973a) and humans inhaling TCI (Cole et al., 1975), and has been shown to be produced in the isolated rat liver upon perfusion with TCI (Bonse et al., 1975) as well as with rat liver microsomal preparations (Byington and Liebman, 1965; Ikeda et al., 1980; Miller and Guengerich, 1982). Defalque (1961) has reviewed extensively the early literature on TCI.

The principal site of metabolism of TCI is the liver, although Dalbey and Bingham (1978) have demonstrated that the rat and guinea pig lung also metabolize TCI to TCE, and TCA. Other tissues, such as kidney, spleen and small intestine, may also metabolize TCI, as these tissues have been shown to be sites of cellular protein binding of metabolites from TCI (Bolt and Filser, 1977; Banerjee and Van Duuren, 1978; Stott et al., 1982).

In addition to the major end-product metabolites of TCI, chloral, TCE, TCE-glucuronide and TCA, a number of other metabolites have been demonstrated in urine or exhaled breath (CO, CO_2, monochloro- and dichloroacetic acids, TCA-glucuronide, chloroform) or *in vitro* with microsomal systems (TCI-epoxide, glyoxylic acid, CO). These metabolites are listed in Table 4-7. Except for dichloroacetic acid and TCA-glucuronide, these metabolites have been established by more than one investigator. However, chloroform, which may be an artifact of analytical methodology, needs further confirmation.

TCI-epoxide (1,1,2-trichloroethene oxide) is known to decompose in aqueous physiological conditions (Table 4-7) to formic acid, dichloroacetic acid, glyoxylic acid and CO. These metabolites may therefore be formed at cellular microsomal sites of TCI-epoxide formation.

4.4.2 Magnitude of TCI Metabolism: Evidence of Dose-Dependent Metabolism

4.4.2.1 Man--Estimates of the extent of metabolism in man, as a percentage of retained TCI, have been made from balance studies, i.e., accounting for a retained dose after vapor exposure by measuring pulmonary elimination of TCI and metabolites excreted into the urine. Balance studies with isotopically labeled TCI have not been reported in man. Man appears to metabolize inhaled TCI extensively (between 40 to 75 percent of the retained dose) as reported by various investigators over the last three decades (Barrett et al., 1939; Powell, 1945; Forssman and Holmqvist, 1953; Soucek and Vlachova, 1960; Bartonicek, 1963; Ogata et al., 1971; Ertle et al., 1972; Muller et al., 1972, 1974, 1975; Kimmerle and Eben, 1973a,b; Stewart et al., 1974; Fernandez et al., 1975, 1977; Vesterberg et al., 1976; Nomiyama and Nomiyama, 1971, 1974a,b, 1977; Sato et al., 1977; Monster et al., 1976, 1979). These studies do not present any evidence to indicate that a saturation of metabolism occurs in man. The data of Nomiyama and Nomiyama (1977) (Figure 4-5) and of Ikeda (1977), which correlate urinary metabolite excretion with increasing levels of TCI exposure, indicate that liver capacity for metabolism of TCI is nonlimiting, at least up to an exposure of 315 ppm (1695 mg/m^3) for 3 h (equivalent to 25 mg TCI/kg b.w.). Furthermore, the alveolar to inspired air concentration ratio (25 percent; retention 75 percent) at exposure equilibrium approximates the cardiac output flow through the liver (25 percent) and suggests that TCI is completely removed from blood by the liver in a single pass. However, Feingold and Holaday (1977) predicted from mathematical simulation models of TCI at anesthetic concentration in man (2000 ppm; arterial concentration, 9.9 mg/dl) that metabolism was rate-limited by saturation to 49 percent of uptake for an 8-h exposure.

TABLE 4-7. DEMONSTRATED METABOLITES OF TCI (OTHER THAN CHLORAL AND CHLORAL DERIVATIVES; TCE, TCE-GLUCURONIDE, AND TCA)

Metabolite	System	Investigator
TCI-epoxide	rat, mouse microsomes	Miller and Guengerich, 1982
Glyoxylic acid	rat, mouse microsomes	Miller and Guengerich, 1982
Carbon monoxide	rat, mouse microsomes	Miller and Guengerich, 1982 Traylor et al., 1977 Fetz et al., 1978
Carbon dioxide	exhaled air rat and mouse	Parchman and Magee, 1982 Stott et al., 1982
Monochloroacetic acid	human and rabbit urine	Soucek and Vlachova, 1960 Ogata and Saeki, 1974
Dichloroacetic acid	mouse urine	Hathaway, 1980
TCA-glucuronide	chimpanzee urine	Muller et al., 1982
Chloroform	rat adipose tissue, serum, urine	Pfaffenberger et al., 1980 Muller et al., 1974
Products from TCI-epoxide decomposition in aqueous solution, at 37°C		
Formic acid		Henschler et al., 1977, 1979
Dichloroacetic acid		Kline and Van Duuren, 1977
Carbon monoxide		Bonse et al., 1975
Glyoxylic acid		Miller and Guengerich, 1982

The problems encountered with balance studies in man are the difficulties associated with accurate measurement of the retained dose of TCI from vapor exposure, and the imprecision of the older methodologies for determinations of urinary metabolites by extraction and colorimetry using modifications of the Fujiwara reaction. More recent careful studies, using GC methods, show that after single or repeated daily exposures from 50 to 380 ppm, an average of 11 percent of retained TCI is eliminated unchanged by the lungs ($t_{1/2}$, 5 h), 2 percent of the dose is eliminated as TCE by the lungs ($t_{1/2}$, 10 to 12 h), and 58 percent is eliminated as urinary metabolites (Fernandez et al., 1975, 1977; Muller et al., 1977; Monster et al., 1976, 1979). Unaccounted for is nearly 30 percent of the TCI dose. These investigators suggest additional pathways or routes of elimination of one or more unknown metabolites. Other reported but unconfirmed minor urinary metabolites of TCI, such as chloroform (1 percent in rats, Muller et al., 1974) and monochloroacetic acid (4 percent in man, Soucek and Vlachova, 1960) do not account for the discrepancy. On the other hand, early studies in dogs by Owens and Marshall (1955) show that TCE-glucuronide is concentrated and secreted in

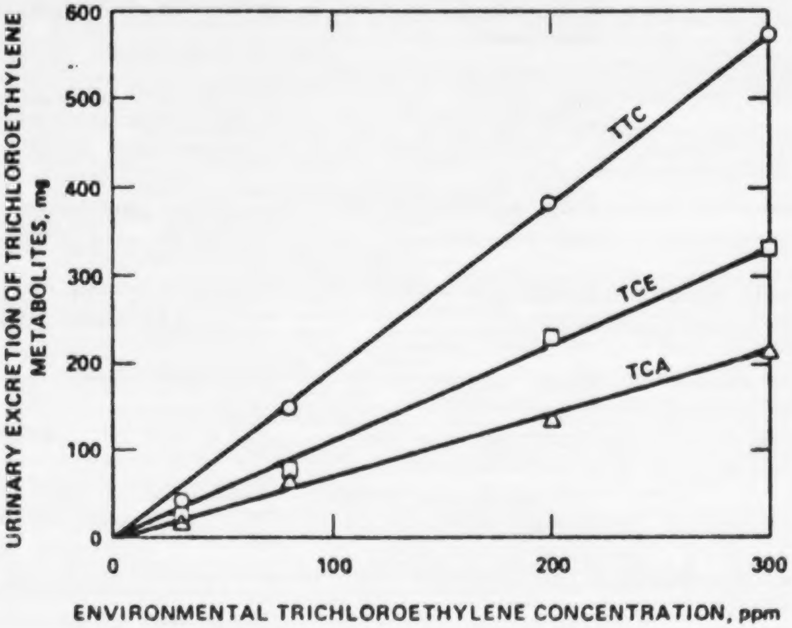


Figure 4-5. Relationship between environmental TCI concentration and urinary excretion of TCI metabolites in human urine. Urine was collected for 6 days after exposure. (Adapted from Nomiyama ad Nomiyama, 1977).

bile. Several studies also show that TCE or TCA given orally or intravenously is not fully recovered in the urine (Marshall and Owens, 1954; Owens and Marshall, 1955; Paykoc and Powell, 1945; Muller et al., 1974). Bartonicek (1962) found that 8.4 percent of a TCI dose is excreted in the feces as TCE and TCA. Part of the unaccounted TCI dose may also be explained by gastrointestinal excretion; TCI can be expected to be rapidly distributed into the GI lumen because of its high lipid solubility and ubiquitous distribution. In any case, these studies indicate at least 60 percent and possibly as much as 90 percent of retained TCI from vapor exposures of less than 500 ppm (2690 mg/m_3) is metabolized in the body.

4.4.2.2 Animals--Early studies by Butler (1948) demonstrated the extensive metabolism of TCI in dogs for which he estimated the extent of metabolism (as a percentage of the administered dose) to be 60 to 90 percent. Muller et al. (1982) recently compared the metabolism of TCI in chimpanzees, baboons and rhesus monkeys. ^{14}C-TCI was administered by intramuscular injection at a dose level of 50 mg/kg. Radioactivity excreted in urine and feces (as an index of metabolism) ranged from 40 to 60 percent of the dose in chimpanzees, 11 to 28 percent in baboons, and 7 to 40 percent in rhesus monkeys. Their results showed a significant difference in excretion route between man/chimpanzee and rhesus/baboon: while man and chimpanzee excreted only from 2 to 5 percent of the totally eliminated TCI metabolites in the feces, baboon and rhesus monkey excreted 18 to 35 percent of the radioactivity in the feces.

For rodents (rat and mouse), the extent of metabolism after single doses of TCI has been estimated by balance studies using isotopically labeled TCI. Daniel (1963) carried out the earliest study in rats. The rats were dosed by stomach tube with ^{36}Cl-labeled TCI liquid (40 to 60 mg/kg b.w.); 80 percent of the dose was eliminated via the lungs with a half-time of elimination of about 5 h; urinary metabolites TCE, TCE-glucuronide and TCA accounted for only 15 percent of the dose. More recent studies show considerably different results.

Stott et al. (1982) exposed rats and mice to 10 and 600 ppm (54 and 3228 mg/m^3) ^{14}C-TCI for 6 h. For exposed mice, virtually 100 percent of the body burden was metabolized with no evidence of metabolic saturation. For rats, however, 98 percent of the body burden from 10 ppm exposure was metabolized but only 79 percent at 600 ppm (3228 mg/m^3) exposure, suggesting a saturation of metabolism in this species. In absolute amounts, mice metabolized 2.2 times more TCI per kg body weight than rats after exposure to 10 ppm, and 3.6 times more after exposure to 600 ppm (3228 mg/m^3) (Tables 4-4 and 4-5).

Filser and Bolt (1979) and Anderson et al. (1980) have also presented strong evidence of saturation of metabolism in the rat (Section 4.2).

4.4.3 Enzyme Pathways of Biotransformation

Current knowledge of the details of the enzymatic biotransformation of TCI derives mainly from *in vitro* studies, principally with liver cell fractions. Figures 4-6 and 4-7 summarize the probable pathways involved in the metabolism of TCI as determined from those studies. Among the halogenated hydrocarbons, TCI biotransformation appears to be unusual, since neither dehalogenation nor removal of carbon atoms occurs in the first step. The initial biotransformation of TCI involves metabolism to chloral hydrate and to formation of trichloroethylene epoxide (Figure 4-6). The formation of chloral/epoxide is the rate-limiting step in the metabolism of TCI (Ikeda et al., 1980; Nomiyama and Nomiyama, 1979). Intramolecular rearrangement with migration of a chlorine atom is presumed to occur in the formation of chloral hydrate, in order to explain the genesis of TCE and TCA. Conversion of chloral hydrate to trichloroethanol (TCE) and trichloroacetic acid (TCA) is shown in Figure 4-7.

4.4.3.1 Formation of chloral--Byington and Leibman (1965) conclusively identified chloral hydrate as the product of TCI when incubated with rat liver microsomes fortified with NADPH and O_2 (P_{450} mono-oxygenase system), thereby verifying the hypothetical chloral hydrate formation proposed by Butler nearly 20 years earlier. These workers also postulated an intermediate formation of an epoxide (as did Powell in 1945), analogous to the known microsomal oxidation to epoxides of dihydronaphthalene, aldrin, heptachlor and isodrin. Daniel (1963) provided evidence that chloride migration must occur in chloral formation since no dilution of ^{36}Cl atoms with chlorine atoms from other body pools was observed in the conversion of ^{36}Cl-TCI to its end products TCE and TCA. Microsomal P_{450}-mediated chloral formation from TCI has been repeatedly confirmed (Ikeda et al., 1980, Miller and Guengerich, 1982) and the formation of the epoxide (1,1,2-trichloroethene oxide) has also been directly demonstrated in liver microsomal preparations by trapping with p-nitrobenzyl pyridine (Miller and Guengerich, 1982). In addition, a specific P_{450} binding spectrum (Type I) in liver microsomes of rats and rabbits has been observed after addition of TCI or TCI-epoxide (Kelley and Brown, 1974; Uehleke et al., 1977b; Pelkonen and Vaino, 1975). TCI also competitively inhibits the metabolism of hexobarbital and aniline by microsomal fractions (Kelley and Brown, 1974), and the magnitude of the binding and chloral formation is enhanced

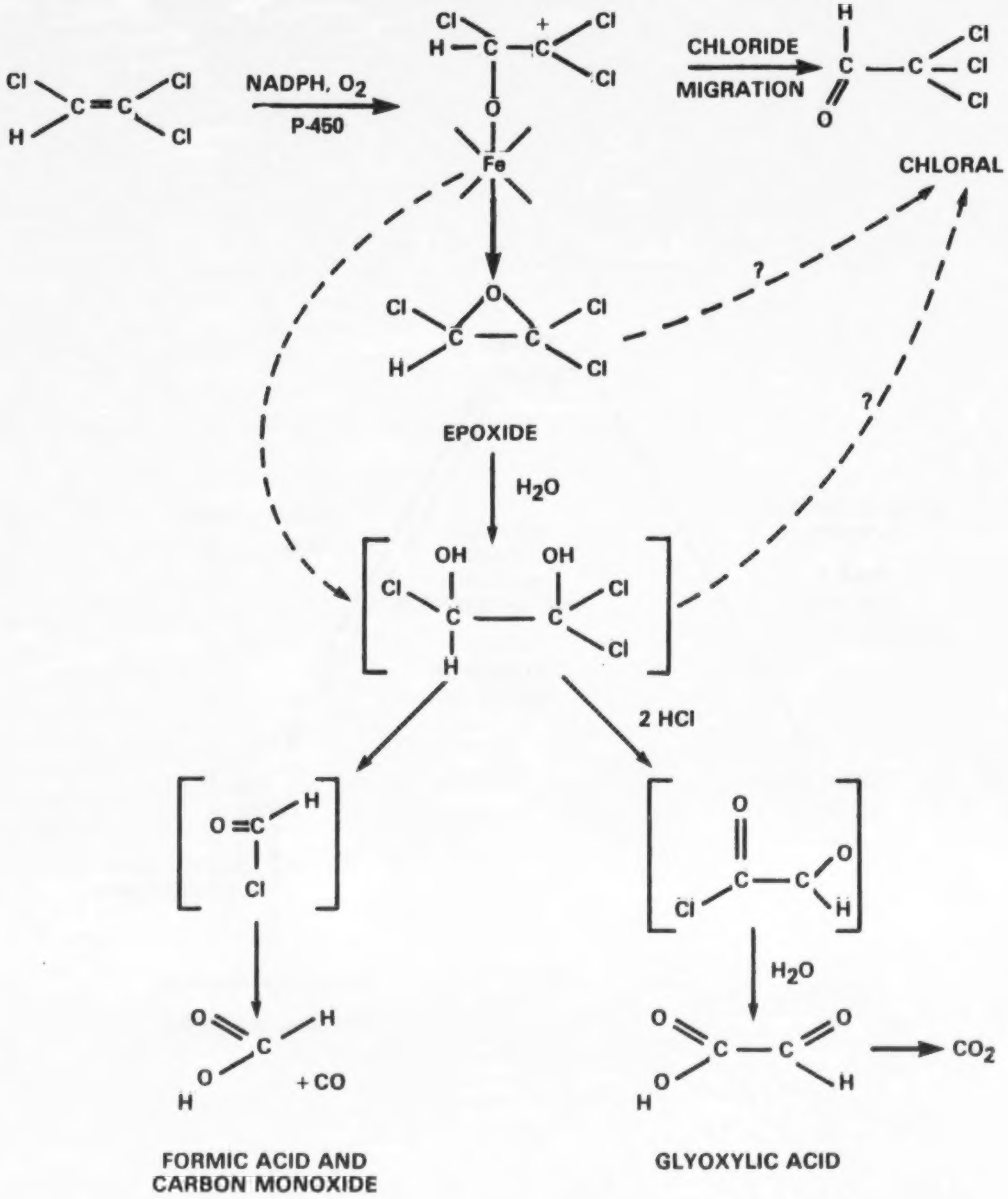

NADPH, O_2
P-450
CHLORIDE
MIGRATION
CHLORAL
?
?
EPOXIDE
H_2O
2 HCl
H_2O
CO_2
+CO
FORMIC ACID AND
CARBON MONOXIDE
GLYOXYLIC ACID

Figure 4-6. Postulated scheme for the metabolism of TCI to chloral (see Figure 4.7 for chloral metabolism) and to trichloroethylene epoxide and its metabolites. (From Miller and Guengerich, 1982).

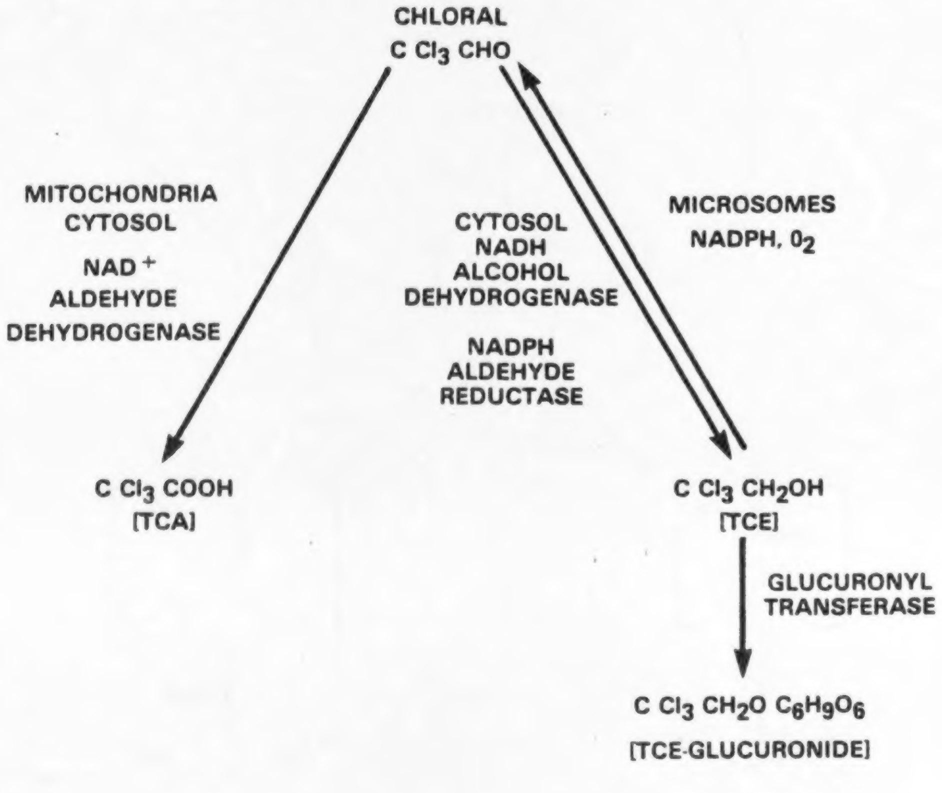

CHLORAL
C Cl3 CHO
MITOCHONDRIA
CYTOSOL
NAD+
ALDEHYDE
DEHYDROGENASE
CYTOSOL
NADH
ALCOHOL
DEHYDROGENASE
NADPH
ALDEHYDE
REDUCTASE
MICROSOMES
NADPH, O2
C Cl3 COOH
[TCA]
C Cl3 CH2OH
[TCE]
GLUCURONYL
TRANSFERASE
C Cl3 CH2O C6H9O6
[TCE-GLUCURONIDE]

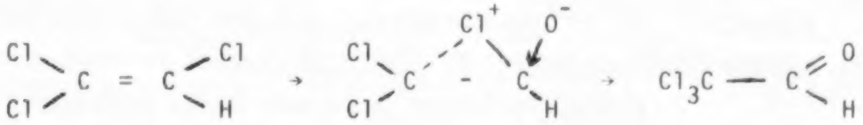
Figure 4-7. Metabolism of chloral hydrate. Modified from Ikeda et al., 1980.

with microsomes from animals pretreated with phenobarbital but not 3-methylcholanthrene (Pelkonen and Vaino, 1975; Leibman and McAllister, 1967). However, the question arises whether the epoxide is an obligatory intermediate in the formation of chloral (Miller and Guengerich, 1982).

Attempts have been made to demonstrate chloral as a metabolite of TCI-oxide. TCI-oxide has been synthesized and its reactions studied under a variety of conditions ($t_{\frac{1}{2}} \sim 1.3$ min in water at pH 7.4, 37°C) Bonse et al., 1975; Kline and Van Duuren, 1977; Henschler et al., 1979; Miller and Guengerich, 1982). Henschler et al. (1979) reported that, under physiological conditions (Tris buffer, pH 7.4), TCI-oxide rearranged to dichloroacetic acid, CO, formic acid and glyoxylic acid, but not to chloral. Entirely similar results were observed by Miller and Guengerich (1982). However, Bonse and Henschler (1976) demonstrated that the TCI oxide can be forced to rearrange to chloral by the catalytic action of Lewis acids ($FeCl_3$, $AlCl_3$, BF_3), and Henschler et al. (1979) proposed that the formation of chloral *in vivo* could therefore be due to catalytic action of the iron of P_{450} in the trivalent form at the microsomal site of formation of the oxide. Although Miller and Guengerich (1982) found that excess ferric iron salts catalyzed the rearrangement of TCI-oxide to chloral in nonaqueous solutions (CH_2Cl_2 or CH_3CN), chloral was not formed in aqueous media even when iron salts, ferriprotoporphyrin IX or purified cytochrome P_{450}, were present.

In studies with rat or mouse liver microsome fortified with NADPH and O_2 (or a reconstituted P_{450} enzyme system), Miller and Guengerich (1982) found that TCI was metabolized to chloral, TCI-oxide, glyoxylic acid and CO. Traylor et al. (1977) also found TCI converted to CO in microsomal incubations. Addition of TCI-oxide to these systems did not produce chloral, but the oxide disappeared with a $t_{\frac{1}{2}} \sim 17$ sec or, in the presence of rat liver epoxide hydrolase, 9 sec. On the basis of the reaction kinetics of formation of chloral and disappearance of TCI-oxide in microsomal preparations, Miller and Guengerich (1982) proposed that TCI-oxide was not an obligatory intermediate step in the formation of chloral. Alternatively, these investigators postulated an oxygenated TCI-P_{450} transition state leading to two products: chloral (with chloride migration) and separate epoxide formation, as shown in Figure 4-6. The working scheme for the metabolism of TCI as presented in Figure 4-6 accounts for all the microsomal metabolites of TCI. Miller and Guengerich (1982) excluded the likelihood of a rearrangement of TCI-oxide or of TCI glycol to chloral, because although rearrangement to chloral occurred in nonaqueous media in the presence of excess ferric iron salt, the incubation of TCI-oxide with purified P_{450} did not result in rearrangement to chloral. Since in vivo chloral is the primary metabolite of TCI metabolism, then in view of Miller and Guengerich's (1982) postulate, the P_{450}-mediated reaction to chloral may be preferential. The possibility of TCI-epoxide being a secondary, minor product in the metabolism of TCI may account for the lesser mutagenic and oncogenic potential of TCI in comparison to other halogenated ethylenes (Bolt et al., 1982).

Van Dyke (1977) and Van Dyke and Chenoweth (1965) also proposed a direct microsomal oxidation to chloral. TCI conversion to chloral was postulated to involve a chloronium ion transition intermediate state in concert with oxidation, presumably by cytochrome P_{450} system. As illustrated, the mechanism does not require an epoxide intermediate. The oxidative attack forces chloride migration to the adjacent carbon if possible, as in the case of TCI; if not, then the chlorine bonded to the carbon, which is oxidatively attacked, is released (dechlorination).



This mechanism also results in "reactive" intermediates which can covalently bind to cell constituents.

4.4.3.2 Metabolism of chloral hydrate--The enzymatic pathways for the biotransformation of chloral hydrate formed from TCI exposure, to the plasma and urinary metabolites TCE, TCE-glucuronide, and TCA, have received less attention in recent years than the mechanism of chloral formation. Current knowledge of the enzymatic pathways of chloral hydrate biotransformation is outlined in Figure 4-7. Chloral hydrate is very rapidly metabolized in vivo with a half-life of only a few minutes (Marshall and Owens, 1954; Breimer et al., 1974; Cole et al., 1975).

A number of investigators have concluded from in vitro studies that chloral hydrate is a substrate for alcohol dehydrogenase purified from horse liver and rat liver cytosol fractions (Butler, 1948, 1949; Marshall and Owens, 1954; Friedman and Cooper, 1960; Sellers et al., 1972b). By this reaction, with NADH as the required coenzyme, chloral hydrate is reduced to TCE (K_m, 2.7×10^{-3}M, horse enzyme). The reaction is essentially unidirectional, since conversion back to chloral hydrate has not been observed with this enzyme (Sellers et al., 1972b;

Owens and Marshall, 1955; Friedman and Cooper, 1960). Tabakoff et al. (1974) studied the reduction of chloral to TCE and postulated the presence of enzymes in rat liver cytosol and the presence of enzymes other than alcohol dehydrogenase that are capable of reducing chloral. Ikeda et al. (1980) demonstrated at least two NADPH-dependent enzymes to be present in rat liver cytosol (in addition to NADH-dependent alcohol dehydrogenase) with a K_m of 6.0×10^{-3}M. These investigators also found that rat liver microsomes in the presence of NADPH and O_2 oxidized TCE to chloral similarly to the oxidation of methanol and ethanol to acetaldehyde (Liebler and DiCarli, 1969).

Human red cells also reduce chloral hydrate to TCE (K_m, 4.0×10^{-4}M) (Sellers et al., 1972b). The efficiency of red cells and their relatively large mass may explain the nearly undetectable levels of chloral hydrate in plasma and urine after exposure to TCI, while TCE is prominent in plasma and urine. TCE-glucuronide present in plasma and urine is accepted as resulting from conjugation by hepatic microsomal glucuronyl transferase.

The origin of TCA as a plasma and urinary metabolite of chloral hydrate is not as clearly defined. The most likely reaction for the oxidation of chloral hydrate to TCA would involve the well-known enzyme acetaldehyde dehydrogenase. However, chloral hydrate has been reported not to be a substrate for human acetaldehyde dehydrogenase (Kraemer and Deitrich, 1968; Blair and Bodley, 1969; Sellers et al., 1972b). Cooper and Friedman (1958) obtained from rabbit liver acetone powder a partially purified form of an NAD-dependent "chloral hydrate dehydrogenase" which was substrate-specific for chloral hydrate. Acetaldehyde was not a substrate but rather a markedly effective inhibitor. Chloral dehydrogenase has not been identified in any other species even though the rabbit, in comparison with the rat or man, poorly converts TCI to TCA. Furthermore, Cooper and Friedman (1958) reported that their enzyme was not inhibited by disulfiram. However, orally administered disulfiram is known to markedly decrease excretion of TCA and TCE and TCI exposure to man (Bartonicek and Teisinger, 1962) and to dogs and rats (Forssman, 1953, cited in Soucek and Vlachova, 1960).

In contrast to earlier studies on aldehyde dehydrogenase that found most of this enzyme activity in the cytosol (Büttner, 1965), more recent studies show that about 80 percent of total activity resides in the mitochondrial and microsomal cellular fractions. Tottmar et al. (1973) described at least two different aldehyde dehydrogenases (NAD and NADP-dependent) in rat liver, localized respectively in mitochondria and microsomes. Grunett (1973) found the NAD-dependent mitochondrial enzyme to have a broad substrate specificity, but chloral hydrate was not a substrate. However, Ikeda et al. (1980) found that an aldehyde dehydrogenase prepared from rat liver mitochondria (NAD-dependent) converted chloral to TCA (K_m, 62.5 x 10^{-3}M). Furthermore, rat liver mitochondria had the highest specific activity for TCA formation, cytosol less, but the microsomal fraction contributed little to the formation of TCA.

Present evidence indicates that the liver is the principal organ contributing to the metabolism of TCI to TCE and TCA. For example, Bonse et al. (1975) showed that TCI perfused through the isolated rat liver led to the appearance of TCA, TCE and chloral in the post perfusate. However, other tissues may also contribute significantly. Dalbey and Bingham (1978) have demonstrated the metabolism of TCI by the isolated perfused lungs of rats and guinea pigs to TCE and TCA, and pretreatment of rats with phenobarbital increased the lung metabolism to TCE. Tabakoff et al. (1974) found that rat brain cytosol was capable of reducing chloral to TCE. The conversion of chloral in the presence of NADPH was 5 times faster than with NADH, the co-factor for alcohol dehydrogenase. As noted above, human red blood cells actively reduce chloral to TCE.

4.4.4 Metabolism and Covalent Binding

The extent of irreversible or covalent binding of reactive intermediates from the metabolism of TCI to cellular macromolecules (protein, RNA, DNA) has been extensively investigated, both *in vitro* and *in vivo*, as a theoretical basis for evaluating the mutagenic and carcinogenic potential of TCI (Bolt et al., 1982). The microsomal oxidative metabolism of TCI to chloral, as outlined in Figure 4-6, suggests the possible reactive metabolites that may result in covalent binding include chloral, TCI-epoxide, dichloroacetyl chloride and formyl chloride. Chloroform, a putative metabolite as yet of indeterminate importance in TCI metabolism, is known to generate covalent binding (Davidson et al., 1982). The derivative metabolites of chloral (TCE, TCE-glucuronide, TCA) have not been implicated in covalent binding. Primary concern accrues to the highly reactive epoxide formed in TCI metabolism.

Henschler and coworkers (Bonse et al., 1975; Greim et al., 1975; Bonse and Henschler, 1976; Henschler and Bonse, 1979) suggested that all chlorinated ethylenes are initially transformed by microsomal P_{450} systems to epoxide intermediates which are subsequently "detoxified" by molecular rearrangement (in the case of TCI to chloral). Further, reactivities, and hence, toxicities of individual epoxide intermediates depend on the type of chlorine substitutions, in that symmetric substitution renders the epoxide relatively stable and not mutagenic, but asymmetric substitution causes unstable and therefore mutagenic epoxides. To explain the lower-than-predicted mutagenic and carcinogenic potential of TCI (Chapter 7), these investigators suggest that TCI is an exception to this general rule, because though its epoxide is asymmetrically substituted and reactive, it immediately upon formation rearranges within the hydrophobic premises of the P_{450} site to the less reactive chloral; thus the epoxide is isolated from reaction with cellular macromolecules and protected from aqueous spontaneous decomposition to other reactive metabolites (dichloroacetyl chloride, formyl chloride) (Henschler et al., 1979). Alternatively, Miller and Guengerich (1982) have provided evidence that the epoxide is not an obligatory intermediate for chloral formation, and that, indeed, the two are separate P_{450} oxidative products with chloral production four- to five-fold greater than the epoxide. Furthermore, they observed a four- to five-fold greater production of chloral plus epoxide from mouse microsomes than from rat microsomes.

4.4.4.1 In vitro Binding--Table 4-8 lists investigators who have demonstrated covalent binding of TCI to microsomal protein nucleic acids and lipids *in vitro*. In many of these experiments, *in vitro* covalent binding from TCI microsomal metabolism was decreased by animal pretreatment with SKF-525A (which inhibits P_{450} metabolism) or enhanced by phenobarbital and other inducers of the P_{450} system. Binding was also decreased by addition of an inhibitor of epoxide hydratase (trichloropropane epoxide) and by the addition of competing nucleophiles such as reduced glutathione, imidazole or mercaptoethanol, indicating the importance of cellular SH groups and glutathione specifically for providing a "detoxification mechanism" for reactive intermediates from TCI metabolism. These experiments provide ample evidence that TCI is metabolized *in vitro* to reactive products by P_{450} systems. Metabolism and covalent binding of reaction products occurs with liver microsomes, the major site of metabolism, but also with microsomes from kidney, stomach, and lung (Banerjee and Van Duuren, 1978). Mice microsomes are two- to four-fold more active in metabolizing TCI to reactive intermediates than rat microsomes, and males greater than females (Uehleke and Poplawski-Tabarelli, 1977; Banerjee and Van Duuren, 1978). DiRenzo et al. (1982a) found that aging rats (27 mo. old) have less capacity for microsomal metabolism, as reflected by covalent binding of TCI, than either adult (11 mo. old) or young (4 mo.) rats. These investigators have also compared the rat

TABLE 4-8. IN VITRO COVALENT BINDING OF TCI

Investigator	System	Macromolecules covalently bound	Additions
Van Duuren and Banerjee (1976)	rat liver microsomes	microsomal protein	SKF-525A or 7,8 benzoflavone- ↓ binding; GSH, methylmercaptoimidazole, mercaptoethanol, urea-↓ binding; epoxide hydratase inhibitor (trichloropropane epoxide) ↑ binding; pretreatment with phenobarbital- ↑ binding
Bolt and Filser (1977) Bolt et al. (1977)	rat liver microsomes	albumin, polylysine	GSH, ↓ binding
Uehleke and Poplawski-Tabarelli (1977)	rat and mice liver microsomes	microsomal protein lipid	Mouse > rat; pretreatment with phenobarbital, ↑ binding
Allemand et al. (1978)	rat liver microsomes	microsomal protein	GSH or piperonyl butoxide - ↓ binding; pretreatment of animals with phenobarbital - ↑ binding
Banerjee and Van Duuren (1978)	Mice (B_6C_3F) and rat (Osborne-Mendel) microsomes	microsomal protein	Mice > rats, males > females; microsomes from all organs bind equally
		Salmon sperm DNA	Male > female enhanced by phenobarbital and 3-methylcholanthrene admin; enhanced by addition of epoxide hydratase inhibitor (trichloropropane epoxide)
Laib et al. (1979)	rat liver microsomes	yeast RNA microsomal protein	VC > TCI TCI > VC
DiRenzo et al. (1982a)	rat liver microsomes	calf thymus DNA	Rats pretreated with phenobarbital
	rat liver microsomes young (4 mo) vs adult (11 mo) vs aged (27 mo)	microsomal protein	↓ P_{450} system in aged; ↓ binding; pretreatment with phenobarbital abolishes difference

hepatic microsomal covalent binding to added DNA for a series of aliphatic halides, including TCI. The degree to which TCI and other aliphatic halides form a DNA-adduct is summarized in Table 4-9. TCI was bioactivated and covalently bound to DNA at a level of 0.36 nmol/mg DNA, or fifth in the series of 10 compounds. Binding was confirmed by sedimentation of the DNA-adduct in a cesium gradient and Sephadex LH-20 chromatography of the nucleotides, although structural identification of modified DNA bases was not made.

4.4.4.2 In vivo binding: A number of investigators have determined the extent of irreversible binding to cellular macromolecules following the administration of TCI to rats and mice. Bolt and Filser (1977) exposed rats (male Wistar) for 5 h in a closed system to initial concentrations of 100 and 1000 ppm (538 and 5380 mg/m^3) ^{14}C-TCI and determined protein-bound metabolites in various organs. The irreversibly bound metabolites were calculated as a percentage of that amount of TCI that was taken up by the animals in 5 h. Radioactivity following exposure was mainly concentrated in the liver (0.77 - 0.88 percent dose), the primary organ of metabolism, and in kidneys (0.37 - 0.39 percent dose), the organ of primary excretion. Lesser amounts were found in lung, spleen, small intestine, and muscle. No major differences in percentage of the dose bound for the different initial atmospheric concentrations of TCI. However, the extent of binding in liver and kidney for TCI was less than for vinyl chloride and approximated that observed with carbon tetrachloride.

TABLE 4-9. MICROSOMAL BIOACTIVATION AND COVALENT BINDING OF ALIPHATIC HALIDES TO CALF THYMUS DNA

Aliphatic halides	Binding to DNA (nmol/mg/h ± SD)
1,2-Dibromoethane	0.52 ± 0.14 (6)
Bromotrichloromethane	0.51 ± 0.18 (6)
Chloroform	0.46 ± 0.13 (6)
Carbon tetrachloride	0.39 ± 0.08 (6)
Trichloroethylene	0.36 ± 0.14 (7)
1,1,2-Trichloroethane	0.35 ± 0.07 (7)
Dichloromethane	0.11 ± 0.05 (5)
Halothane	0.08 ± 0.01 (6)
1,2-Dichloroethane	0.06 ± 0.02 (6)
1,1,1-Trichloroethane	0.05 ± 0.01 (3)

^{14}C-labeled aliphatic halides (1 mM) were incubated with hepatic microsomes. Carbon tetrachloride, bromotrichloromethane and halothane were incubated under an N_2 atmosphere, and all other incubations were under an O_2 atmosphere. From DiRenzo et al., 1982b.

Uehleke and Poplawski-Tabarelli (1977) determined _in vivo_ binding for mice (NMRI) following intraperitoneal injections of ^{14}C-TCI (0.2 μCi/10 μmole in 5 μl of peanut oil/g b.w.). Binding to live microsomes, mitochondria, and cytosol protein was measured at 2, 6, 12, and 24 h after injection. Peak binding occurred after 6 h (8, 4 and 1.8 nmoles/mg protein) respectively, and thereafter declined slowly. When the same dose of ^{14}C-TCI was given by gavage at the 6-h time, binding to microsomal protein was found to be only 5.4 nmole/mg protein. ^{14}C-TCI was also bound to hepatic lipids; similarly to protein binding, 6 h after i.p. dosage, the highest concentrations were 9.4 nmole/mg of endoplasmic fractions of livers and 5.6 nmole/mg in mitochondrial lipids of the liver.

Allemand et al. (1978) investigated the metabolic activation of TCI and irreversible binding in Sprague-Dawley rats after ^{14}C-TCI (100 μmol, 100 μCi) was administered i.p. in 200 μl of methanol to nonpretreated and to phenobarbital-pretreated rats; the rats were sacrificed 4 h later. Trace amounts of irreversibly bound radioactivity were found on muscle proteins (3.1 mmole equiv./g tissue), while 40 times more was found on liver proteins (117 nmole equiv./g tissue). Pretreatment of the animals with phenobarbital did not significantly increase muscle binding but increased liver binding by 46 percent. Furthermore, TCI reduced hepatic glutathione levels 61 percent 4 h after administration, confirming similar observations by Moslen et al. (1977b).

Parchman and Magee (1982) compared _in vivo_ covalent binding of TCI metabolites to liver protein and DNA in B6C3F1 male mice and Sprague-Dawley male rats. Rats were injected i.p. with 10, 100, 500, and 1000 mg/kg b.w. of ^{14}C-TCI in corn oil (each animal received 160 μCi/kg of radioactivity). After 6 h, liver "cytoplasm" and "nucleus" fractions contained 2.5 to 6.1 and 0.3 to 3.9 percent of dose radioactivity, respectively. Radioactivity was detected in DNA isolated from the nucleus fraction and purified by cesium chloride centrifugation and extraction with phenol, isoamyl alcohol and chloroform. However, the level of radioactivity was extremely low (35 cpm/mg DNA) and contamination with labeled protein could not be entirely ruled out. Identification of DNA adducts by hydrolysis followed by HPLC of nucleotides was not successful. Similar results were obtained with mice injected i.p. with 10 and 250 mg/kg ^{14}C-TCI (160 μCi/kg b.w.) and sacrificed 6 h later. Hepatic DNA contained only 51 cpm/mg for the lower dose and 283 cpm/mg for the higher dose. Compared to DNA binding by dimethylnitrosamine (DMN) under identical conditions, the carcinogen-binding index (Lutz, 1979) calculated for TCI was only 2, as compared to 2045 for DMN.

These investigators concluded that the ability of TCI to interact with DNA in vivo was very slight.

Stott et al. (1982) investigated covalent binding of TCI in male B6C3F1 mice and male Osborne-Mendel rats, the rodent strains used for carcinogenicity testing of TCI by the National Cancer Institute (NCI) (NCI, 1976). The animals were exposed to 10 or 600 ppm (54 or 3228 mg/m^3) of ^{14}C-TCI (11.3 mCi/nmol) for 6 h. The mice metabolized more inhaled TCI than rats at low and high exposure levels (123 and 260 percent, respectively). The amount of hepatic and renal covalent binding was three-fold higher in mice than for the high dose (Table 4-10) and four-fold greater for liver than kidney. Mice (4) were also dosed with 1200 mg/kg of ^{14}C-TCI with relatively high specific activity (2.4 mCi/nmol) and a maximum estimate of a liver DNA alkylation level of 0.62 ± 0.42 alkylations/10^6 nucleotides was observed after isolation and separation by HPLC (Table 4-11). However, no chemical identification of possible nucleotide adducts was made. Maximum covalent binding index observed was 0.12, or one-twentieth of that estimated by Parchman and Magee (1982), and many orders of magnitude less than that for known carcinogens such as DMN, methylnitrosurea, etc. (footnote, Table 4-11). These results are in accord with the conclusion of Parchman and Magee (1982) that the ability of TCI to interact with DNA in vivo is very slight.

Stott et al. (1982) also chronically administered to male B6C3F1 mice (by gavage) 2400 mg TCI/kg/day for 3 days. Localized cell necrosis, enhanced DNA synthesis, and centrilobular hepatocellular swelling was observed. With prolonged exposure (3 weeks), the primary response observed was dose-related centrilobular hepatocellular swelling and the occurrence of mineralized cells. In Osborne-Mendel male rats, a maximum tolerated dose of 1100 mg/kg/day led to increased liver weight and elevated DNA synthesis not coupled to histopathology, but consistent with a simple increase in the number of normal hepatocytes. In view of the weak genotoxic potential of TCI, as indicated by only slight DNA covalent binding and weak hepatotoxicity in mice, these investigators suggest an epigenetic mechanism for the tumorigenic action of TCI observed in this species only in the NCI carcinogenicity bioassay (NCI, 1976). They implicate also the greater covalent protein binding (three-fold) that occurs in the mouse versus the rat from the three-fold greater total metabolism of TCI per kilogram body weight.

TABLE 4-10. HEPATIC AND RENAL MACROMOLECULAR BINDING OF 1,1,2 (UL-^{14}C) METABOLITE IN MALE B6C3F1 MICE AND OSBORNE-MENDEL RATS EXPOSED TO 10 OR 600 PPM FOR 6 HOURS. From Stott et al. (1982)

Exposure (ppm)	Tissue	Test animal	Binding (pmol-eq(^{14}C) TCI/μg protein) ± SD	Ratio (mouse/rat)
10	Liver	Mouse	0.318 (0.004)	1.2
		Rat	0.268 (0.013)	
	Kidney	Mouse	0.168 (0.014)	1.1
		Rat	0.155 (0.007)	
600	Liver	Mouse	20.4 (2.49)	4.3
		Rat	4.72 (0.415)	
	Kidney	Mouse	5.06 (0.667)	3.0
		Rat	1.77 (0.200)	

TABLE 4-11. *IN VIVO* ALKYLATION OF HEPATIC DNA BY 1,1,2(UL-^{14}C) TCI IN MALE B6C3F1 MICE DOSED WITH 1200 MG/KG (^{14}C) TCI BY GAVAGE. From Stott et al. (1982)

Animal	Detection limit (alk/10^4)	Non-C_1 dpm[a] > 2 Sigma error	Maximum alkylations 10^4 nucleotides[b]	Maximum CBI[c]
1	0.12	18.0	0.50	0.055
2	0.52	ND[d]	--	--
3	0.15	7.1	0.27	0.030
4	0.18	26.6	1.1	0.120

[a] dpm not associated on HPLC analysis with normal DNA bases from normal metabolic incorporation and not attributable to counting error (i.e., mean background dpm + 2 SD).

[b] "Maximum" alkylations possible due to the relatively large amount of dpm associated with protein binding and metabolic incorporation into normal bases.

[c] Covalent binding index (Lutz, 1979). Comparative hepatic CBI for dimethylnitrosamine, diethylnitrosamine, methylnitrosourea, and aflatoxin B-1 are approximately 5500, 125, 640, 17,000, respectively. CBI = (μmol adduct/mol dN) (nmol/kg dose).

[d] None detected.

4.4.5 TCI Metabolism: Drug and Other Interactions

A number of commonly used drugs might be expected to modify the extent of metabolism of TCI during human exposure. The converse may also occur, resulting in important modifications of the therapeutic action of drugs. Although largely undocumented in man, the induction of hepatic microsomal mixed-function oxidate system by drugs taken for therapeutic reasons or by exposure to certain environmental chemicals (e.g., barbiturates, PCBs, etc.), may bring about an increased rate of TCI metabolism. Numerous *in vivo* and *in vitro* experimental studies with animals pretreated with microsomal inducers have demonstrated an enhanced metabolism of TCI (Leibman and McAllister, 1967; Carlson, 1974; Moslen et al., 1977a,b; Pessayre et al., 1979). Conversely, TCI competitively inhibits the metabolism of barbiturates, producing exaggerated effects of these drugs (Kelley and Brown, 1974).

4.4.5.1 Disulfiram--A drug interaction has been shown to occur in persons exposed to TCI who are also on a therapeutic regimen of disulfiram for alcoholism. Disulfiram inhibits the oxidation of acetaldehyde by competing with NAD for active sites on aldehyde dehydrogenase. Bartonicek and Teisinger (1962) showed that disulfiram markedly inhibits the terminal enzymatic steps of TCI metabolism in man. Since these steps, which involve the conversion of chloral hydrate to TCE and TCA (Figure 4-7), can be considered detoxification mechanisms, disulfiram has the potential of enhancing TCI and chloral hydrate toxicities.

4.4.5.2 Alcohol--Sellers et al. (1972b) investigated the interaction of ethanol and chloral hydrate metabolisms. These investigators observed that concomitant administration of alcohol and chloral hydrate in man exacerbated the side effects of chloral hydrate (vasodilatation, tachycardia, facial flushing, headache and hypotension). Their patients also exhibited impairment of auditory function and of performance of complex motor tasks. Similar effects have been observed during acute and chronic TCI exposure and concomitant ingestion of alcohol (Muller et al., 1975). Stewart et al. (1974) have described the phenomenon of "degreasers" flush (facial and upper extremity skin vasodilatation) and determined that this effect of alcohol interaction with TCI persists after cessation of exposure. This intolerance to alcohol syndrome is ascribed to mutual inhibition of TCI and alcohol metabolisms producing increased plasma ethanol and TCE concentrations from competitive inhibition of aldehyde dehydrogenase (Sellers et al., 1972a,b; Stewart et al., 1974).

Muller et al. (1975) specifically investigated the interaction of TCI and alcohol in human volunteers. After the volunteers inhaled TCI (50 ppm) for 6 h per day for 5 days, ethanol ingestion inhibited TCI metabolism to TCE and TCA by 40 percent. Blood TCI concentration increased more than two-fold. This latter observation suggested that alcohol also inhibited, by competition, the microsomal oxidation of TCI to chloral hydrate. These investigators proposed that the intolerance syndrome from combined exposure to TCI and ethanol was due to increased accumulation of TCI in the CNS, resulting from depression of TCI oxidation. From experiments in rats, Nakanishi et al. (1978) found that ethanol ingested after TCI exposure increased blood acetaldehyde levels three- to four-fold. They suggest that TCI inhibits acetaldehyde dehydrogenase, and the resulting increase of blood levels of acetaldehyde is responsible for the intolerance syndrome.

White and Carlson (1981), working with rabbits, observed that acute ethanol administration (1 g/kg i.v. or p.o. 30 mm prior to TCI exposure, 6000 ppm; 32280 mg/m^3) decreased TCI metabolism, as indicated by increased peak blood levels of TCI (22 percent), decreased peak blood levels of TCE (50 percent) and decreased blood levels of TCA (99 percent). Also, the zero-order metabolism of alcohol itself was decreased 25 percent by TCI exposure (blood ethanol disappearance rate, -0.707 control vs. -0.539 exposed, mg/dl/min). Furthermore, rabbits treated with ethanol exhibited markedly increased susceptibility to the development of TCI-induced cardiac arrhythmias, provoked by epinephrine. The results of this ethanol study in rabbits confirm those of the human studies by Muller et al. (1975).

In contrast to these studies, Sato et al. (1981) found that an acute ethanol dose to rats (4 g/kg, 40 percent solution intragastric) significantly lowered the blood concentration decay curve of TCI resulting from a subsequent (16 h later) 400 ppm (2152 mg/m^3) for 4-h exposure to TCI. Furthermore, ethanol-treated rats excreted in urine much greater amounts (three-fold) of total trichloro compounds (TCE and TCA) than untreated rats. These findings would indicate that a single large dose of ethanol can accelerate the *in vivo* metabolism of TCI. When ethanol (4 g/kg) was given to rats and liver microsomes were prepared and tested for their rate of metabolism of TCI, an enhancement of TCI metabolic rate was found, with the greatest increase (two-fold) occurring 16 h after ethanol dosing. At this time, almost no blood ethanol was detected and no increase occurred in microsomal protein and P_{450} liver contents. Ethanol addition directly to the TCI microsomal incubations inhibited TCI metabolism, thus demonstrating that ethanol was a competitor of TCI metabolism. These investigators suggest that ethanol, when taken *in vivo*, can exert a dual effect on drug-metabolizing enzymes, i.e., both inhibition and stimulation of TCI metabolism. When present in an early period at high blood levels, ethanol inhibits metabolism of TCI; but ethanol also increases metabolic potential of the enzymes so that 16 to 18 h later, when ethanol blood level has dissipated, the microsomal enzymes have an increased capacity to metabolize TCI.

4.4.5.3 Warfarin: Sellers and Koch-Weser (1970) observed a potentiation of the anticoagulant effect of Warfarin in patients after chloral hydrate ingestion. This increased potential for bleeding appeared to result from displacement of Warfarin from plasma protein binding sites by the chloral hydrate metabolite, TCA. Considering the significant amounts of TCA formed after TCI exposure and the long half-time of excretion of TCA, it is likely that TCI may potentiate the effects of many other drugs which normally bind to the same plasma protein sites as TCA.

4.4.5.4 Diet and age: Nakajima et al. (1982) have explored the effect of diet on the hepatic microsomal metabolism of TCI and other chlorinated hydrocarbons. These investigators found that hepatic microsomes from rats fed for 3 weeks on an isocaloric diet deficient in carbohydrate (sucrose) had an increased capacity (two and one half-fold) to metabolize TCI and other chlorinated hydrocarbons. Varying protein content of the diet produced only a negligible influence on enzyme activity. Microsomal protein and P_{450} hepatic content (per g liver) were not significantly changed, although liver weight was slightly decreased. They suggest from their findings that a low-carbohydrate diet produces enhanced P_{450} system activity. This concept, according to these investigators, is not in conflict with the widely held belief that a high-protein diet enhances this activity, because high-protein diets are usually low in carbohydrate to maintain isocaloric conditions. Thus, a diet rich in carbohydrate may offer significant protection against the hepatic toxicity of chlorinated compounds by decelerating their metabolism.

DiRenzo et al. (1982a) have recently shown that the hepatic microsomal capacity to metabolize TCI and other chlorinated hydrocarbons is influenced by chronological age. These investigators isolated hepatic microsomes from young (4 mo.), adult (11 mo.) and aging (27 mo.) Fischer 344 rats. Biotransformation and subsequent covalent binding of labeled TCI and other compounds was studied. Levels of microsomal protein and lipid binding increased slightly between 4 and 11 months, but decreased 50 to 75 percent in the 27-month-old animals, as compared to young adults. Senescent rats were also found to have significantly less hepatic P_{450} (-35 percent), NADPH cytochrome C reductase (-31 percent), and ethylmorphine N-demethylase activity (-43 percent). Pretreatment with phenobarbital, however, abolished these deficiencies of aging rats. These investigators suggest a reduced metabolic capacity as a function of age, which results in a decrease in bioactivation of the aliphatic halides, and hence, possible differences in toxicity in the elderly from xenobiotics requiring bioactivation.

4.4.6 Metabolism and Cellular Toxicity

The metabolism of TCI has been suggested as a possible vector for the hepatotoxicity and nephrotoxicity associated with excess human exposure to this haloalkene (James, 1963; Defalque, 1961; Smith, 1966; Litt and Cohen, 1969; Baerg and Kimberg, 1970). Experimentally, TCI has been shown to be hepatotoxic in dogs and rats (Orth and Gillespie, 1945; Klaassen and Plaa, 1967; Cornish and Adefuin, 1966; Carlson, 1974; Cornish et al., 1977; Reynolds and Moslen, 1977). Reynolds and Moslen (1977) and Henschler and Bonse (1979), on physicochemical and biological correlative bases, have proposed that unsymmetric chlorinated ethylenes like TCI are more hepatotoxic than symmetric ethylenes as perchloroethylene and more so than vinyl chloride. The mechanism for TCI hepatotoxicity is not definitely established, but many investigators have proposed that the observed centrilobular cellular necrosis results from the microsomal mixed-function oxidase system formation of chemically "reactive" metabolites which react with and bind to tissue macromolecules and cause intracellular damage (Pessayre et al., 1979; Allemand et al., 1978; Reynolds and Moslen, 1977). Figure 4-6 indicates that these reactive metabolites may include trichloroethylene epoxide, dichloroacetyl chloride and formyl chloride (Section 4.4.4). Chloral, chloral hydrate and trichloroethanol, as ordinarily used hypnotics, are not associated with significant hepatotoxicity (Goodman and Gilman, 1975).

Presently, there is little information about the degree of binding of "reactive" metabolites to macromolecules that results in a toxic liver response; nor have the critical target binding sites been identified. However, numerous factors, such as species, age, sex, and diet, all of which modify TCI microsomal metabolism, also markedly influence the toxic response. Drugs such as phenobarbital (Carlson, 1974) and ethanol (Cornish and Adefuin, 1966) greatly influence TCI metabolism and hepatotoxicity. Cornish et al. (1977) found that TCI-induced liver toxicity in rats, as measured by plasma SGOT, is potentiated by both ethanol and phenobarbital. Ethanol acted by competitive substrate inhibition, and also by stimulation of microsomal mixed-function oxidase system; phenobarbital potentiated toxicity by induction of mixed function oxidase system and thereby increased TCI metabolism. Allemand et al. (1978) observed a similar potentiation of toxicity in rats, as measured by plasma SGPT. Plasma SGPT increase correlated with the amount of binding o. ^{14}C-TCI reactive metabolites to liver microsomal protein. Reduced glutathione had a protection effect against TCI liver toxicity. TCI acutely administered to rats initially decreased hepatic glutathione by 61 percent, but increased glutathione levels (106 percent) 16 h later (Moslen et al., 1977b). Glutathione added to microsomal preparations decreased binding of reactive products of ^{14}C-TCI, suggesting a glutathione conjugation of metabolites. Pessayre et al. (1979) found that single or acute TCI administration to rats decreased cytochrome P_{450} of phenobarbital-treated rats, but not untreated animals, and increased covalent binding of ^{4}C-TCI metabolites to microsomal protein. Chronic administration to normal rats simultaneously decreased P_{450} cytochrome content and increased ^{14}C-TCI metabolite binding, but also induced other microsomal enzyme systems. Since acute TCI exposure rarely produces functional liver impairment in man (Smith, 1966), whereas chronic exposure does (Litt and Cohen, 1969; Baerg and Kimberg, 1970), Pessayre and co-workers (1979) suggest that the explanation may lie in the ability of TCI to enhance its own metabolism and its own toxicity.

Poplawski-Tabarelli and Uehleke (1982) have investigated other mechanisms that contribute to the toxic manifestations of halogenated aliphatic hydrocarbons, including TCI. These investigators attempted a correlation of the inhibition by these compounds of various microsomal oxidative reactions. Of the several possible factors influencing these mono-oxygenase activities, such as interaction with lipid environment of microsomal cytochromes; ligand formation with reduced P_{450} cytochrome; covalent interactions of metabolic intermediates with cytochrome P_{450}; destruction of cytochromes by lipid peroxidation; and production and interaction of CO with cytochromes, the overriding correlation appeared with the simple physicochemical factors of high lipid solubility and vapor pressure. The ability of chlorinated ethylene to inhibit mitochondrial respiration and hence ATP production as a mechanism of cellular toxicity has also been investigated. Takano and Miyazaki (1982) found that all of a series of 14 chlorinated ethanes and ethylenes, including TCI, inhibited oxygen consumption of isolated rat mitochondria; their inhibitory potency increased in the order of the number of contained chlorines.

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5. TOXICOLOGICAL EFFECTS IN MAN AND EXPERIMENTAL ANIMALS

5.1 INTRODUCTION

Trichloroethylene (TCI) has been an important industrial chemical since its commercial application in 1906. It has been the focus of wide research interest and the subject of numerous case reports. The available literature concerning the potential effects of TCI is reviewed in this chapter.

5.2 EFFECTS ON THE CENTRAL NERVOUS SYSTEM

5.2.1 Human Studies: Acute Inhalation Exposure

The first extensive study of the effects of TCI on the nervous system was that of Stuber in 1931. Stuber reviewed a total of 283 cases of presumable overexposure to TCI, including 26 fatalities. All cases had occurred in European industrial operations. Stuber reported that the action of TCI primarily involved the central nervous system (CNS), although some effects were also observed in the gastrointestinal and circulatory systems. Adverse effects on the kidney were rare, and no cases were reported of injury to the liver. Among the CNS symptoms most often described were headache, dizziness, vertigo, tremors, nausea, vomiting, sleepiness, fatigue, a feeling and appearance of lightheadedness or "drunkenness" increasing to unconsciousness, and, in some cases, to death. In addition to the general narcotic effect, specific paralysis of the trigeminal nerve was also reported (Plessner, 1915; Persson, 1934). This observation led to the early use of TCI in the treatment of trigeminal neuralgia.

Numerous case histories have subsequently been reported on the effects of acute exposure to high doses of TCI (Longley and Jones, 1963; Masoero and Lavarino, 1955; Vallee and Leclercq, 1935). The earlier studies reported on the toxicity of TCI following anesthesia, in particular the occurrence of trigeminal palsies (Humphrey and McClelland, 1944). Boulton and Sweet (1960) suggested that trigeminal palsies that occurred in 24 patients resulted from inhalation of dichloroacetylene or phosgene during closed-circuit TCI anesthesia. These compounds are formed when exhaled TCI is passed through soda lime to remove carbon dioxide. The formation of these impurities and their subsequent toxicity have resulted in a decline in the use of TCI as an anesthetic.

Kleinfeld and Tabershaw (1954) reported on five fatal cases of TCI poisoning among industrial workers following acute overexposure. This was the first compilation of TCI-related fatalities. Four of the workmen, who had been employed in a degreasing operation, continued to work despite complaints of nausea, drowsiness, dizziness, and vomiting. All died while at work or within a few hours after leaving work. Death was attributed to ventricular fibrillation.

St. Hill (1966) reported the case of a man who died following acute exposure to TCI while boiling out tanks in a ship's hold after an accidental leakage. The man was exposed for about 20 h, during which time he complained of headache, dizziness, double vision, and paralysis of the face and neck muscles. Two other men who had worked with the victim suffered similar but less severe symptoms. Similar symptoms were reported by Tomasini and Sartorelli (1971) in a patient, following chronic and acute overexposure to TCI during operation of a dry cleaning unit. The symptoms included symmetrical bilateral VIIIth cranial nerve deafness as well as cerebral cortical dysrhythmia and alterations in the electroencephalogram (EEG). This patient recovered after exposure was stopped.

Loss of special senses has also been reported following TCI exposure. Mitchell and Parsons-Smith (1969) reported the case of a worker who lost his sense of taste after one month's exposure to high concentrations of TCI. He subsequently developed trigeminal analgesia and cortical changes reflected in alterations in the EEG.

Maloof (1949) reported blurring of vision and diplopia in a worker the day after he had been exposed to hot TCI vapors. In addition to symptoms characteristic of visual effects and effects on the CNS, the worker received first- and second-degree burns to the skin and first-degree chemical burns to the eyes.

Steinberg (1981) associated acute exposure to high levels of TCI with permanent CNS damage in a 62-year-old man. Damage persisted six years following exposure.

Lawrence and Partyka (1981) reported an acute overexposure to TCI which resulted in chronic involvement of the bulbar cranial nerves and esophageal and pharyngeal motility impairment.

Many other such case histories of acute TCI exposure have been reported with similar symptomatologies. Visual disturbances, mental confusion, fatigue, nausea, and vomiting have all been observed. The dangers of acute exposure to TCI may be accentuated by visual disturbances and by loss of coordination. This may lead to poor manual dexterity and unsafe mechanical operations (Patty, 1958).

Trichloroethylene has also been reported to have some abuse potential. Cases of deliberate and repetitive inhalation have been reported by James (1963) and by Ikeda and Imamura (1973). Abuse of TCI has been associated with hepatorenal toxicity (Clearfield, 1970). Respiratory and cardiac failure is believed to be the cause of death after acute inhalation exposure.

5.2.2 Human Studies: Chronic Exposure

Occupational exposure reports generally do not provide important information that is needed to relate effects to exposure levels. Often the degree of exposure is not well defined. Other factors often missing or given limited coverage in such reports are (1) contamination with other noxious substances, (2) existing pathological conditions in those exposed.

Chronic exposure to levels of TCI greatly in excess of those found or expected in ambient air results primarily in neurological and neuropsychiatric symptoms consisting of tremors, giddiness, increased lacrimation, reddening of skin, decreased sensitivity of the hands, alcohol intolerance, neuroanesthetic syndrome with anxiety states, bradycardia, and insomnia (Bardodej and Vyskocil, 1956). Other manifestations are not as well defined. The first published report of chronic occupational overexposure to TCI in the United States summarized ten individual cases occurring prior to 1944 (McNally, 1937; Quadland, 1944). The symptoms in all the cases were characteristic of CNS disturbances. No quantitative estimation of exposure levels was provided.

Kunz and Isenschmid (1935) reported the case of a worker who used TCI to remove diamond powder from the rolls of a jewelry roll. It was estimated that 100 to 300 ml of the solvent evaporated directly in front of his face daily. Blindness was preceded by increasing changes in color perception and vision.

Loss of tactile sense, decreased mobility, and the inability to grasp objects between the thumb and fingers was noted in six women working with TCI to remove wax remaining on optical lenses (Albahary et al., 1959).

5.2.3 Experimental Human Studies

5.2.3.1 Controlled Studies--Psychomotor function and subjective responses to TCI have been studied in short-term, controlled, human clinical studies to determine the effects of TCI on the CNS. Kylin et al. (1967) measured optokinetic nystagmus in 12 human subjects after exposure to 1000 ppm (5380 mg/m^3) TCI for 2 h. Although a significant decrease in the fusion limit was noted, this test was found not to be a sensitive indicator of the effects of TCI. Stopps and McLaughlin (1967) exposed one subject to four different concentrations of TCI (100, 200, 300, and 500 ppm; 538, 1076, 1714, and 2690 mg/m^3). Two experiments were run at each level, with concentrations presented in ascending and descending order. Each experiment was 3 h in duration. They found no significant effect on psychomotor performance at 100 ppm (538 mg/m^3), but they found a progressive decrease in performance as the TCI concentration was increased. This study, however, was limited by the use of only one subject and by the limited exposure-period used in the experiment, which is not comparable to an 8-h work day. In a larger study involving six male students exposed to two 4-h exposures in one day at an average concentration of 110 ppm (592 mg/m^3), Salvini et al. (1971) demonstrated a statistically significant decrease in performance ability for tests of perception, manual dexterity, reaction time, and Wechsler memory scale.

The effects of TCI on visual motor performance, as determined by psychophysiological tests, were studied in 12 subjects by Vernon and Ferguson (1969). They found a significant decrease in performance only at the highest concentration tested (1000 ppm; 5380 mg/m^3) after a 2-h exposure.

Stewart et al. (1970) experimentally exposed ten subjects to 100 and 200 ppm (1076 mg/m^3) TCI for periods ranging from 1 h to a 5-day work week. They reported mild fatigue and sleepiness in normal adults after 4 to 5 days' exposure to 200 ppm (1076 mg/m^3) TCI for 7 h per day. Nomiyama and Nomiyama (1977) reported headaches in healthy male students exposed to about 100 ppm (538 mg/m^3) TCI for 4 h per day for 6 days. No effects were observed on a flicker fusion or two-point discrimination test even at 200 ppm (1076 mg/m^3).

In a pilot study to determine the effect of TCI exposure on mental capacity, Ettema et al. (1975) found no evident or consistent effect on task performance or on physiological parameters. Fourteen adult males were exposed for 2.5 h to 0(n = 11), 75(n =11) or 300(n = 11) ppm. The tests used were described by the authors as requiring active cooperation and exertion from the subjects; it was cautioned that the results could not be extrapolated to vigilance situations.

The short duration of many of these exposures decreases the value of these studies in assessing the longer-term effects of TCI on nervous system function. They are useful primarily in measuring the threshold level for general CNS-depressant activities of TCI.

5.2.3.2 Epidemiological Studies--Several epidemiological studies have been conducted in an attempt to correlate environmental concentrations and health effects. Grandjean (1963) found that 73 workers, exposed for 5 to 15 years to TCI concentrations of 1 to 333 ppm (5.38 to 1791 mg/m^3), exhibited alterations in autonomic nervous system function that were related to the length of time they were exposed to TCI; these changes were also accompanied by a high frequency of subjective symptoms. Bardodej and Vyskocil (1956) reported insomnia, tremors, and severe neuroasthenia syndromes coupled with anxiety states and bradycardia in 75 workers divided into four groups according to number of years they were exposed to TCI (5 to 629 ppm; 27 to 3384 mg/m^3). The groups were exposed less than 1 year, 1 to 2 years, 2 to 9 years, and over 10 years. They found that the symptoms increased in frequency with both longer exposures and higher concentrations of TCI. Disturbances of the nervous system were reported to continue for up to 1 year after final exposure. As Messite (1974) reported, these studies did not include control groups and were based primarily on clinical impressions, rather than statistical analyses.

In a detailed study of 104 workers exposed to TCI in metal, rubber, and dry cleaning industries, Andersson (1957) reported that two-thirds of the workers displayed CNS symptoms similar to those described for acute toxicity. Follow-up studies of the workers for 3 to 7 years after exposure showed little residual evidence of TCI intoxication. Although the average exposure level in this study was 200 to 400 ppm (1076 to 2152 mg/m^3), it should be noted that the levels of exposure were subject to many unmeasurable fluctuations.

Lilis et al. (1969) reported a study of 70 young workers (83 percent less than 30 years old) exposed for up to 6 years to variable concentrations of TCI. The subjects showed symptoms of CNS and cardiac disturbances. Although the authors estimated the TCI concentrations to be about 10 ppm (54 mg/m^3), a direct relationship between the incidence and severity of symptoms and the duration of exposure was not established.

Konietzko et al. (1978) reported changes in EEGs that correlated with subjective toxic effects reported by workers exposed to open basins of TCI. Studies such as these are somewhat limited and thus provide only suggestive information. Due to lack of background data and the potential interaction of other chemicals, it is difficult to attribute the observed effects directly to TCI exposure.

Szulc-Kuberska et al. (1976) reported occupational exposure of TCI workers resulted in impairments of the auditory and vestibular nerves. Forty workers were examined, ranging in age from 25 to 50. Exposure levels were not reported nor was the test group compared to a control population. Because the individuals were reported to have had at least 40 mg TCI/l urine, it can be concluded that exposure levels were exceedingly high.

5.2.4 Animal Studies

5.2.4.1 Nervous System and Behavior--There have been few in-depth studies of the effects of TCI on the nervous system and behavior. Studies apparently have not been performed on such behaviors as learning or extinction, accuracy or efficiency of complex schedule-controlled behaviors, or sensory discriminations. Most endpoints that have been evaluated were studied in only a preliminary manner. Nervous system electrophysiology, neurochemistry, and neuropathology are areas that warrant more attention.

Taylor (1936) found no significant serious adverse effects in rats exposed to 500, 1000, and 2000 ppm (2690, 5380, and 10,760 mg/m^3) TCI for 6 h/day, 5 days/week, for 6 months. At 3000 ppm (16,140 mg/m^3) only two of 6 mice survived. Dogs exposed to 2000 ppm (10,760 mg/m^3) TCI and guinea pigs exposed over 1100 h to 1200 ppm (6456 mg/m^3) TCI showed no adverse effects.

Baker (1958) investigated the histological changes in the CNS associated with acute and chronic exposure of dogs to TCI. The study was designed to produce graded exposures to TCI experimentally. Although no neuropathological changes could be detected after a single acute exposure to 30,000 ppm (161,400 mg/m^3) TCI, repeated exposure led to brain changes at scattered loci. Chronic exposure of dogs (2 to 8 h/day, 5 days/week) to 300 to 500 ppm (1614 to 2690 mg/m^3) TCI resulted in selective destruction of the cerebellar Purkinje cells. Similar findings were reported by Bartonicek and Brun (1970) in rabbits chronically injected intramuscularly with a total of 53 g of TCI. The most pronounced changes appeared to occur in the Purkinje cells.

Tham et al. (1979) studied the effect of TCI on the vestibular system in rabbits by recording positional or rotational nystagmus. They found that at blood levels greater than 30 mg/l (following continuous i.v. infusion of 1 to 5 mg/kg/min), TCI, unlike chloral hydrate and trichloroethanol, induced positional nystagmus. Their results suggested that TCI elicited vestibular disturbances by stimulating the central subcortical vestibulo-oculomotor connection.

During the development of a potential animal model for solvent abuse, Utesch et al. (1981) found that high levels of TCI, from 9000 to 16,000 ppm (48,420 to 86,080 mg/m^3), adversely affected CNS functioning in Wistar-Munich rats but produced no signs of kidney or liver damage. Performance measures used were the loss of the righting reflex and performance on a tilting plane. Inhalation of 9000 ppm (48,420 mg/m^3) for up to 15 min caused only slight difficulty in locomotion. All of a group of 6 rats lost their righting reflex after approximately 5 min at 14,000 ppm (75,320 mg/m^3). Characteristic signs at 14,000 ppm (75,320 mg/m^3) included irritation of mucous membranes of the eyes and respiratory tract, hyperpnea, and progressive ataxia. Performance on the tilting plane was used to evaluate recovery. All animals appeared to be fully recovered within 15 min after cessation of exposure to 15,000 ppm (80,700 mg/m^3). Groups of 5 or 6 rats subjected to 15,000 ppm (80,700 mg/m^3) for 5-min periods, with 15-min solvent-free intervals between subsequent exposures (total = 1-5 h), did not show any elevations in SGOT or SGPT nor were there any adverse histopathological findings. Influence of ethanol on effects of repetitive TCI inhalation is described in Section 5.7.

Behavioral techniques have also been used to study the effect of TCI and its metabolites in animals. Grandjean (1960) studied the effects of TCI on food-motivated conditioned responses in three rats. Exposure was via inhalation of 200 and 800 ppm (1076 and 4304 mg/m^3) TCI for 3 h. He was unable to detect obvious behavioral changes related to TCI exposure, although there was some evidence of increased excitability or loss of inhibition. Battig and Grandjean (1963) chronically exposed rats to 400 ppm (2152 mg/m^3) TCI for 10 months (8 h/day, 5 days/ week). Although exposure to TCI did not adversely affect their general condition, their swimming speed was reduced. They also found a significant increase in exploratory behavior, which confirmed their earlier studies (Grandjean, 1960). In a later study, Grandjean (1963) exposed rats for 6 h to 400, 800, and 1600 ppm (2152, 4304, and 8608 mg/m^3) TCI. Although only slight decrements in swimming behavior were observed after 400 and 800 ppm (2152 and 4304 mg/m^3), 1600 ppm (8608 mg/m^3) produced significant decreases. This was also accompanied by evidence of fatigue at the higher dose.

Goldberg et al. (1964a) trained four rats in a conditioned avoidance escape test (pole climbing). Four-hour exposures, 5 days/week for 2 weeks to 200, 500, and 1500 ppm (1076, 2690, and 8070 mg/m^3) TCI did not result in altered responses. Learning appeared to be stimulated by 200 ppm (1076 mg/m^3)

exposure, and higher doses (4380 ppm; 23,564 mg/m^3) produced a specific inhibition of the avoidance response with no accompanying sign of motor imbalance. The responses did not increase or decrease directly with dose level. In a subsequent study, Goldberg et al. (1964b) evaluated further the effects of TCI on behavioral responses in the CFE rat. Discrete avoidance studies were performed in a 10-compartment rat-shock box. Ten rats had been trained to 95-percent or higher efficiency in avoiding shock. Once trained, the rats retained a stable baseline for many months. The shock-box was placed in the exposure chamber. Each vapor study was composed of three phases: pre-exposure control, vapor exposure, and post-exposure control. Rats stayed in the chamber 4 h a day, 5 days/week, for weeks. Exposure concentration was 128 ± 11 ppm (689 ± 59 mg/m^3). During 25 exposure days, a significant increase in the number of shocks taken by the group was observed on 22 days. No significant effect was observed during the first day of each of the first 3 weeks of study. Effects were reversible during the recovery phase. Silverman and Williams (1975), using observational methods, showed that repeated exposure (6 to 7 h/day, 5 days/week, 4 weeks) of rats to 100 ppm (538 mg/m^3) TCI produced slight behavior changes in these animals.

The neurotoxicological effects of TCI on rodents have been demonstrated by Haglid et al. (1981). Biochemical changes and ultrastructural evidence of neuronal cell alterations were found in various areas of the brain of adult Mongolian gerbils exposed continuously to 60 or 320 ppm (323 or 1722 mg/m^3) for 3 months, followed by a period of 4 months free of exposure. The most pronounced changes were in the hippocampus, the posterior part of the cerebellar vermis, and in the brain stem. Biochemical findings indicated a glia cell reactivity in some brain areas, which the investigations believed was caused by a primary effect of TCI or its metabolites or secondary to damage to neuronal cells. In affected areas, total soluble proteins were decreased, while the S-100 fraction, a glial cytoplasmic protein, was significantly elevated, as measured per soluble protein. In sensory-motor cortex and hippocampus, elevations of S-100 protein per soluble protein after exposure were larger following inhalation of 60 ppm than after 320. The investigators concluded that observed alterations could serve as early and sensitive measurements of toxic effects of organic solvents on the CNS. The observations on glial brain-specific protein in discrete brain areas confirmed previous work by these investigators (Haglid et al., 1980). Previously, it had been demonstrated that soluble proteins in various areas of the brain of gerbils exposed to TCI for 8 weeks (320 ppm; 1722 mg/m^3) were altered differently. The authors cautioned that the biochemical changes could reflect adaptation.

Exposure of NMRI mice to 170 ppm (915 mg/m^3) TCI continuously for 30 days did not affect brain levels of acetylcholinesterase, glutamine synthetase, acid phosphatase and glutamate dehydrogenase activity nor cholinesterase activity in blood. These observations suggested to the authors that nerve cell populations and glial cells were unaffected by TCI (Kanje et al., 1981). In contrast, plasma butyrlcholinesterase increased two-fold in exposed male mice but was not increased in females. Liver weight in males and females increased by factors of 1.5 and 1.9, respectively.

Kjellstrand et al. (1982) tested the possibility that acid phosphatase could be a sensitive detector of brain damage by exposing rats, mice, and gerbils continuously to 150 ppm (807 mg/m^3) TCI for 30 days. The enzyme assay system had a limit of detection of ± 10 to 15 percent. Acid phosphatase levels were measured in five different areas of the brain. Small differences in total enzyme activity and activity per mg brain tissue were seen between the TCI and air-exposed groups in the brain stem of mice ($p < 0.05$) and gerbils ($p < 0.01$). It was concluded that the alterations of acid phosphatase observed were not prominent enough to consider acid phosphatase as a very sensitive measure of brain damage.

5.3 EFFECTS ON THE CARDIOVASCULAR AND RESPIRATORY SYSTEMS

5.3.1 Cardiovascular Effects

The use of TCI as an anesthetic has been associated with cardiac arrhythmias, including bradycardia, atrial and ventricular premature contractions, and ventricular extrasystoles. The dose-response relationships for these effects, however, has not been established in man or experimental animals. High concentrations of TCI have been reported to sensitize the myocardium to circulatory catecholamines, resulting in cardiac arrhythmias including ventricular fibrillation or even cardiac arrest (Dhuner et al., 1951, 1957; Reinhardt et al., 1973). Metabolites of TCI, such as chloral hydrate, may also be associated with arrhythmias involving adrenergic stimulation (DiGiovanni, 1969). Lilis et al. (1969) observed in their study that 60 percent of the workers exposed to inhaled TCI exhibited an increase in cardiac output, an effect attributed to increases of epinephrine release and circulating plasma levels. These direct cardiac effects of TCI, with sensitization to catecholamines, have been observed after accidental ingestion and have, in some cases, proved fatal (Defalque, 1961).

Several case histories have been reported involving fatalities following acute exposure to large concentrations of TCI. The deaths in these cases were attributed to either cardiac arrest (James, 1963) or ventricular fibrillation (Kleinfield and Tabershaw, 1954). Bell (1951), Bardodej and Vyskocil (1956), Ogata et al. (1971), Andersson (1957), and others have noted that exposure to TCI may either speed up or slow down the heart rate, depending on the degree of exposure. The most direct evidence that TCI causes ventricular extrasystoles, fibrillation, and cardiac arrest comes from changes that are observed in the electrocardiograms (EKG) of subjects accidentally exposed to high concentrations of TCI (Kledecki and Bura, 1963; Mroczek and Fedyk, 1971; Yacoub et al., 1973). Bernstine (1954) recorded ventricular fibrillation confirmed by EKG in a worker who sustained cardiac arrest after inhalation of TCI in analgesic concentrations. Barnes and Ives (1944) found that 33 of 40 normal patients in deep anesthetic planes showed signs of ventricular extrasystoles and multifocal ventricular tachycardia.

Few systematic animal studies have been done on the effects of TCI on the cardiovascular system. Mazza and Brancaccio (1967) studied the effects on rabbits of chronic exposures to 2790 ppm (15,010 mg/m^3) TCI, 4 h/day, 6 days/week, for 45 days. They found that severe blood dyscrasia was produced in these animals. The characteristics of the hemochromocytometric tests and the description of the bone marrow led the authors to conclude that chronic intoxication with TCI has a direct effect on the bone marrow, which causes myelotoxic anemia. Mikiskova and Mikiska (1966) studied electrophysiological responses of guinea pigs injected intraperitoneally either with 6.7 mM/kg TCI or with 2.2 mM/kg trichloroethanol. They recorded EKG's and EEG's and found that trichloroethanol was at least three times as effective as TCI in inducing effects on the nervous system and in slowing the heart, and suggested that TCI effects were due, in part, to its metabolite, trichloroethanol.

Caffeine has been shown to potentiate the arrhythmogenicity of TCI in rabbits upon challenge with exogenously-administered epinephrine (White and Carlson, 1982). Rabbits were treated with a vehicle control (1 mg/kg, i.p.) or caffeine (10 mg/kg, i.p. 30 min prior to exposure) and exposed for 1 h to 6000 ppm (32,280 mg/m^3) TCI under dynamic airflow conditions. Epinephrine was infused until arrhythmias occurred after 7.5, 15, 30, 45 and 60 min of exposure and 15 and 30 min post-exposure. The authors reported a pronounced increase in the incidence of epinephrine-induced arrhythmias in TCI-exposed rabbits, when treated with caffeine and challenged with doses of epinephrine as low as 0.5 μg/kg.

White and Carlson (1981) also investigated the effect of ethanol on epinephrine-induced cardiac arrhythmias in rabbits exposed to 6000 ppm (32,280 mg/m^3) TCI. Rabbits were treated with saline (1 ml/kg) or ethanol (1 g/kg, i.v. or p.o.) 30 min prior to exposure to 6000 ppm TCI for 1 h. Epinephrine was infused as described above. Rabbits treated with ethanol developed epinephrine-induced cardiac arrhythmias sooner and at lower doses of epinephrine than control rabbits. More arrhythmias developed in rabbits treated with ethanol i.v. than p.o. Ethanol administration decreased the conversion of TCI to trichloroethanol by 50 percent, and trichloroacetic acid production was completely blocked by ethanol treatment.

Fossa et al. (1982) demonstrated that disulfiram exhibits a cardioprotective activity not related to its effects on TCI metabolism in rabbits. Disulfiram (1.35 mmoles/kg, p.o.) was administered 24 and 6 h prior to a 1-h exposure to 6000 ppm (32,280 mg/m^3) TCI. Disulfiram or its metabolites, CS_2 and diethyldethiocarbamate (1.35 mmoles/kg, i.p.), were administered at similar time intervals also to rabbits exposed for 1 h to 9000 ppm (48,420 mg/m^3) TCI. Blood levels of TCI and its metabolites, trichloroethanol and trichloroacetic acid, were measured at periods ranging from 7.5 min of exposure to 30 min post-exposure. The metabolism of TCI was inhibited in all treatment groups. When challenged with 0.5 to 3.0 μg/kg epinephrine, disulfiram prevented the epinephrine-induced arrhythmias resulting from exposure to 6000 ppm (32,280 mg/m^3) TCI. All treatment groups in the first 30 min of exposure to 9000 ppm (48,420 mg/m^3) TCI exhibited an increased percentage of animals responding with arrhythmias compared to controls. Thirty to sixty minutes of exposure resulted in a significant decrease in the percentage of animals responding with arrhythmias in the disulfiram- and diethyldithiocarbamate-treated groups.

5.3.2 Respiratory Effects

In anesthetic concentrations, TCI causes little or no irritation to the respiratory tract (Dobkin and Byles, 1963). A striking effect is observed, however, on the rate and depth of respiration. TCI characteristically causes increased respiratory rate (tachypnea) but decreased alveolar ventilatory amplitude, which is associated with decreased blood oxygen tension and increased carbon dioxide tension (Dobkin and Byles, 1963). These effects were noted in early animal experiments (Lehmann, 1911) and in early reports on human patients under TCI anesthesia (Striker et al., 1935). The incidence of tachypnea increases as the concentration of TCI increases (Atkinson, 1960).

To investigate the mechanism responsible for the increase in respiratory rate, Whitteridge and Bülbring (1944) studied the effects of TCI on pulmonary afferent nerve endings in cats. They found that 0.5 to 3.5 percent (5,000 to 35,000 ppm; 26,900 to 188,300 mg/m^3) TCI produced a dose-dependent increase in the frequency of vagal discharge and an increased sensitivity of the stretch endings. Coleridge et al. (1968) observed similar results in isolated pulmonary receptor endings.

Stewart et al. (1970) attempted to correlate injury to rat pulmonary alveolar parenchyma with biochemical alterations following repeated inhalation of TCI. Mild changes in morphology were observed, including condensed mitochondria and vacuolation of the cells. Pulmonary surfactant secretion was significantly decreased. The authors suggested that modification of surfactant secretion was a consequence of nonspecific injury following exposure of a susceptible cell population to TCI.

5.4 HEPATIC AND RENAL TOXICITY

5.4.1 Human Studies

The similarity between TCI and chloroform and other related halocarbons known to cause liver damage suggests that TCI might possess hepatotoxic activity. Stuber (1931) reviewed 284 cases of industrial poisoning due to TCI and found no evidence of liver damage. Brittain (1948) found no evidence of liver or kidney damage in 250 neurosurgery patients who underwent prolonged TCI anesthesia. However, fatal acute hepatic failure has been observed following the use of TCI as an anesthetic. Such failure has generally been in patients with other complications such as malnutrition, toxemias and burns, or patients who had received transfusions (Ayre, 1945; Berleb, 1953; Dodds, 1945; Elam, 1949; Heizer, 1951; and Werch et al., 1955).

Secchi et al. (1968) reported acute liver disease in three of seven cases of poisoning following ingestion of TCI. They suggested, however, that the results could be attributed to contamination with 1,2-dichloropropane and 1,2-dichloroethane since no liver damage was associated with ingestion of pure TCI. Cotter (1960) described 10 patients acutely exposed to TCI in a confined space. Four patients showed hyperglobulinemia and six exhibited hypercalcemia. No other liver tests were abnormal and, in all but one person, the clinical tests returned to normal within two months of exposure. Lachnit and Brichta (1958) reported that of 22 workers occupationally exposed to TCI, only three had abnormal reactions on two liver function tests (sulfobromophthalein clearance tests and colloid stability). Albahary et al. (1959) conducted liver function tests--measuring serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT)--on workers regularly exposed to TCI. No evidence of liver disorders was found. Guyotjeannin and Van Steenkiste (1958) found some abnormalities in cephalic flocculation, total lipids, and plasma unsaturated fatty acids, and an increase of plasma gamma globulins in 18 workers regularly exposed to TCI. Tolot et al. (1964) found no evidence of liver injury, even though workers were routinely exposed to TCI at concentrations higher than the allowable tolerance limits, that is, 0.9 to 3 mg of TCI per liter of air (167 to 558 ppm). Joron et al. (1955) reported massive liver necrosis (particularly the left lobe) followed by death of a chemist exposed intermittently to TCI over a 1.5-year period. Subsequent analyses of room air indicated TCI concentrations as high as 300 ppm (1614 mg/m^3).

From the above reports, it is apparent that observations of liver dysfunction in workers exposed to TCI have been infrequent, and relatively little surveillance has been conducted to ascertain latent effects. Thus, it is unlikely that the potential magnitude of any kidney or liver abnormalities in humans exposed for long periods to TCI is fully known. Cases of severe liver and kidney damage resulting from ingestion of liquid TCI may provide some evidence for latent toxicity. However, it is difficult to predict whether the same effects would be observed if ingestion of TCI was spread over a period of time similar to that found in inhalation exposure.

As mentioned earlier, TCI has also been reported to have some abuse potential. Cases of deliberate and repetitive inhalation have been reported by James (1963) and by Ikeda and Imamura (1973). Baerg and Kimberg (1970) documented centrilobular hepatic necrosis and acute hepatic and renal damage following repeated sniffing of cleaning fluid containing TCI and petroleum distillates by teenagers. However, as the vapor concentration was high and inhalation was only for a short period, the effect cannot be directly extrapolated to inhalation of the same total dose over a long period of time, as in occupational exposure.

Thiele et al. (1982) reported that acute overexposure to TCI, as well as methyl chloroform, resulted in a diagnosis of hepatic cirrhosis in a 37-year-old male industrial worker.

5.4.2 Animal Studies

Early experiments showed some degree of liver damage. It should be noted that TCI test mixtures used, especially prior to 1960, likely contained contaminants that may have contributed to the effects observed. With two groups of dogs exposed to 750 ppm (4035 mg/m^3) TCI 8 h/day, 6 days/week, for 3 weeks, and 500 to 600 ppm (2690 to 3228 mg/m^3) TCI 6 h/day, 5 days/week, for 8 weeks, Seifter (1944) found glycogen depletion and hydropic parenchymatous liver degeneration. Wirtschafter and Cronyn (1964) injected rats with TCI subcutaneously (0.5 g/kg); they found elevated SGOT levels within 12 to 16 h, which were correlated with hepatocellular damage observed in histological sections. This suggested a mild degenerative change of liver parenchyma. Gehring (1968) observed no significant increase in SGPT following a single exposure of mice to 5500 ppm (29,590 mg/m^3). Nowill et al. (1954) exposed one dog, three rabbits, and three rats to 0.05 to 0.1 percent (500-1000 ppm; 2690 to 5380 mg/m^3) TCI for 18 h/day for 90 days. No gross abnormality was observed in liver, renal, or blood functions. Utesch et al. (1981) exposed rats intermittently to 15,000 ppm (80,700 mg/m^3) TCI in a manner simulating human solvent abuse. No evidence of liver or kidney damage was observed. Pendergast et al. (1967) reported negative observations following continuous exposure of rats, guinea pigs, dogs, rabbits, and monkeys to TCI. Ikeda et al. (1969) injected rats every 2 days for 37 days (0.5 ml/kg and 1.5 ml/kg). No significant changes were found in liver or blood lipids.

Liver failure in experimental animals is associated with binding of TCI metabolites to protein and nucleic acids in the liver (Bolt and Filser, 1977). Binding of TCI metabolites and TCI hepatotoxicity are increased by compounds that induce the liver mixed-function oxidase system and by treatment with inhibitors of epoxide hydrase. Adams et al. (1951) and Moslen et al. (1977) have found that TCI inhalation does not produce acute liver necrosis unless animals are pretreated with phenobarbital, especially when fasting. In addition, in phenobarbital-treated rats, hepatic glutathione contents decreased during TCI exposure, rebounding afterwards (Moslen et al., 1977). Despite the reports of hepatotoxicity in experimental animals exposed to high doses and in humans exposed to anesthetic doses of TCI, liver damage similar to that observed in human industrial exposure is rare (Bardodej and Vyskocil, 1956) and difficult to produce in experimental animals with acute subanesthetic doses (Kylin et al., 1963).

Danni et al. (1981) also reported that TCI exposure of male Wistar rats did not result in any signs of hepatic injury. Trichloroethylene had been given orally to 5 animals, at a dose of 0.65 mmoles/100 g body weight and to 3 animals at 2.58 mmoles/100 g body weight. Relative liver weight and hepatic triglyceride content were used as parameters in measuring liver injury. Injury was observed only when the rats were first pretreated with phenobarbital.

In a laboratory screening study for relative hepatotoxicity in mice, Jones et al. (1958) reported that the oral administration of TCI in olive oil (0.36 to 4.3 mg/g body weight) to 200 Swiss mice, mixed sexes, in groups of 10, resulted in at least minimal liver injury. The minimal hepatotoxic dose reported was 0.72 mg/g. The principal change noted was fatty infiltration. The minimal lethal dose reported was 2.92 mg/g. Histologic examination of the liver was made 72 h after exposure.

Ramadan and Ramadan (1969) reported that 12 albino rats exposed to anesthetic levels of TCI for 2 h did not show signs of parenchymal liver damage. The distribution of acid phosphatase positive granules in rat hepatic parenchyma was, however, shown to be irregular and their number diminished, 24 h after exposure.

Dow Chemical (Stott et al., 1981) has recently performed gavage studies which include dosages similar to those used in the National Cancer Institute (NCI) (1976) bioassay for carcinogenicity of TCI by gavage in B6C3F1 mice and Osborne-Mendel rats (see Chapter 7). Male B6C3F1 mice were gavaged with 0, 250, 500, 1200, or 2400 mg TCI/kg body weight/day, 5 days a week, for 3 weeks. Male Osborne-Mendel rats, treated similarly, received 0 or 1100 mg TCI/kg body weight/day. Dose-related changes characteristic of hepatocellular hypertrophy were observed at all dosage levels in mice, and ranged from slight histological changes at the lowest dose to increased liver weights at 500 mg/kg, to increased centrilobular hepatocellular swelling at 1200 mg/kg, to severe centrilobular hepatocyte swelling, giant cell inflammation, and mineralized cells at the highest dose. No effect on the kidneys or on body weights was observed in either mice or rats. Rats treated with 1100 mg TCI/kg body weight had slightly increased liver weights but no histopathological changes in the livers. The authors suggest that the greater sensitivity of B6C3F1 mice to the hepatotoxic effects of TCI reflects their greater capacity for metabolic activation of TCI (Section 4.6). From the results of their macromolecular binding experiments, including DNA alkylation studies (Section 4.6), and from these toxicity data, Stott and coworkers (1981) concluded that TCI induction of hepatic tumors in B6C3F1 mice may occur via an epigenetic mechanism.

Renal failure has been an uncommon problem with TCI anesthesia (Defalque, 1961). Although depressed kidney function has been observed in experimental animals exposed to TCI, it requires very high doses (Klassen and Plaa, 1967); on a relative basis, TCI is much less potent than chloroform or carbon tetrachloride.

The acute and subchronic toxicology of TCI in weanling CD-1 and ICR outbred albino mice has been recently investigated by Tucker et al. (1982). The oral LD_{50} in female mice was found to fall in the 95 percent confidence range of 1839 to 3779 mg/kg, compared to 2065 to 2771 mg/kg for males. In a 14-day gavage study, the two doses selected (24 and 240 mg/kg) approximated 1/10 and 1/100 of the LD_{50}. The only effect noted in the 14-day study was an apparently dose-related increase in liver weight. TCI was dissolved in a 10-percent Emulphor solution. In a 5-day study to determine the effect of TCI on hepatic microsomal activities, a group of 11 mice was given TCI according to the following regimen: 0.73 g/kg twice on day 0; 1.46 g/kg twice on day 1, 2.91 g/kg twice on day 3, and 1.46 g/kg on days 4 and 5. Mice were sacrificed on day 6. Activities found to be significantly ($p < 0.05$) elevated included aniline hydroxylase. Aminopyrine N-demethylase was significantly decreased.

In the 4- and 6-month subchronic studies, 140 mice of each sex were administered TCI in drinking water at the following levels: 0.1, 1.0, 2.5 and 5 mg/ml in 1-percent Emulphor. Weight gain was lower for each sex, compared with vehicle group, but was not statistically significant. Decreased water consumption was evident, with females at the highest dose level and with males at the two highest doses, compared with vehicle controls.

Results of gross pathological examinations performed on mice killed at 4 and 6 months were unremarkable. A significant increase in kidney weight occurred at the highest dose in males at 6 months and in females at both 4 and 6 months. Urinalysis indicated elevated protein and ketone levels in high-dose females and the two highest-dose males after 6 months of exposure. Hematology findings included a decreased erythrocyte count in the high-dose males at 4 and 6 months (13 and 16 percent, respectively); decreased leukocyte counts, particularly in females at 4 months; and altered coagulation values consisting of increased fibrinogen in males at both times and shortened prothrombin time in females at 6 months. The observed results suggested to the authors that at doses lower than the LD_{50}, TCI is not a potent hepatotoxin in the mouse.

5.5 IMMUNOLOGY

The immunological status of male and female CD-1 mice exposed to TCI in drinking water for 4 and 6 months was investigated by Sanders et al. (1982). Details of exposure and discussion of toxicological effects noted were reported separately by Tucker et al. (1982) (see Section 5.4). In the 14-day range-finding study, male CD-1 mice had been dosed by p.o. gavage daily with 24 or 240 mg/kg TCI. Although the humoral immune response to sheep RBC was not significantly different from control, the cell-mediated response was inhibited in a dose-related manner. To determine the immune response over longer periods of time, both male and female mice were exposed to TCI (0.1, 1.0, 2.5, and 5 mg/ml) in drinking water for 4 and 6 months. Parameters evaluated included humoral immunity, cell-mediated immunity, lymphocyte responsiveness, bone marrow function, and macrophage function. It was observed that male mice were relatively unaffected after both 4 and 6 months of exposure compared to results in the 14-day study. On the other hand, females were affected, particularly in the 4-month exposure. Humoral immunity was inhibited only at the highest TCI concentrations (2.5 and 5 mg/ml), whereas cell-mediated immunity and bone marrow stem cell colonization were inhibited at all four concentrations of TCI. The proliferation capacity of the lymphocyte was not affected when challenged with T-cell mitogens.

5.6 DERMAL EFFECTS

Industrial use of TCI is often associated with dermatological problems (Bauer and Rabens, 1974). These include reddening and dermatographic skin burns on contact (Maloof, 1949), generalized dermatitis resulting from contact with the vapor (McBirney, 1954), and possible scleroderma (Reinl, 1957). However, irritations, burns, and rashes are usually the result of direct skin contact with concentrated solvent and are, therefore, probably limited to effects secondary to the solvent. Stewart and Dodd (1964), in a study of controlled skin exposure (thumbs), showed that unless TCI is trapped against the skin, absorption is insignificant. Absorption of TCI varies with age, skin thickness and texture, as well as type of contact. No effects have been reported with exposure to TCI in dilute aqueous solutions.

5.7 MODIFICATION OF TOXICITY: SYNERGISM AND ANTAGONISM

The manifestation of adverse effects of TCI appears to depend, in part, on its metabolic products. Therefore, drugs and chemicals that enhance or inhibit the metabolism of TCI can have a corresponding effect on the toxicity of TCI. Reynolds (1976) and Moslen et al. (1977) have reported that the hepatotoxic potential of TCI can be greatly enhanced by prior treatment with drugs or chemicals that induce components of the liver mixed-function oxidase (MFO) system. Carlson (1974) demonstrated that pretreatment of male albino rats with phenobarbital (50 mg/kg i.p.) or 3-methylcholanthrene (40 mg/kg) exacerbated TCI-induced liver damage. There was massive necrosis and a three-fold increase in SGOT and SGPT levels. Groups of animals were exposed to 6900 ppm (37,122 mg/m^3) for 2 h and 10,400 ppm (55,952 mg/m^3) for 2 h. This was confirmed in a later study of Moslen et al. (1977), who found that TCI induced morphological changes in the liver of animals treated with only phenobarbital. Reynolds (1976) also found acute liver injury following a 2-h exposure to 1 percent TCI in animals pretreated with other inducers of MFO, including chlorinated biphenyls (Arochlor 1245). These findings suggest that a metabolite(s) of TCI contributes to the toxicity of the compound. In this regard, Mikiskova and Mikiska (1966) have found that trichloroethanol, a metabolite of TCI, was three to five times more effective in inducing changes in EKG and EEG.

Masuda and Nakayama (1982) found that TCI produced moderate increases in plasma glutamine pyruvate transminase activity in male SPF mice given an i.p. dose of 2 ml/kg (dissolved in olive oil). Diethyldithiocarbamate trihydrate and CS_2, when given to the mice orally, 30 min before TCI, exerted a protective effect, in that the increase in plasma glutamine-pyruvate transaminase was lessened. Diethyldithiocarbamate was effective at each of the three dose levels used (10, 30, and 100 mg/kg). In controls given only TCI, no marked pathological alterations were reported upon histological examinations. CS_2, at levels of 3, 10, and 30 mg/kg, acted similarly to diethyldithiocarbamate in suppressing the TCI-associated increase in plasma glutamate-pyruvate transaminase. The authors concluded that the protective mechanisms of diethyldithiocarbamate and CS_2 may involve their interference with metabolic activation of a variety of hepatotoxins.

Novakova et al. (1981) reported that an open-ended *in vitro* rat liver perfusion system could be used to demonstrate the effect of TCI and other halogenated solvents on liver enzyme activities. TCI in such a system was reported to increase the activities of aspartate aminotransferase, alamine aminotransferase, alkaline phosphatase, and ornithine carbamoyltransferase.

Pessayre et al. (1982) have obtained evidence that TCI is an effective potentiator of hepatotoxic effects of CCl_4 in vivo in rats. While liver histology was normal 24 hours after i.p. administration of TCI (1 ml/kg) and also after administration of 64 µl/kg CCl_4, both solvents coadministered produced extensive centrilobular necrosis. TCI given in vitro 5 h prior to CCl_4 increased CCl_4-induced lipid peroxidation. Similarly, coadministration markedly increased serum alamine amino transferase and decreased hepatic cytochrome P_{450} concentrations. Coadministration also decreased hepatic glutathione levels. It was concluded that the toxicologic effects produced by the concomitant administration of TCI and a small dose of CCl_4 resembled those produced by a high dose of CCl_4, a finding consistent with the view that TCI potentiated the hepatotoxicity of CCl_4. It was further concluded that this potentiation may be the result of increased CCl_4-induced rate of lipid peroxidation. Since stable metabolites of TCI failed to stimulate such peroxidation, it was apparently mediated by TCI itself.

The effect of ethanol and other substances upon the manifestation of effects of subsequent TCI exposure in animals has been studied by a number of investigators.

One of the earliest studies was that of Cornish and Adefuin (1966). Male Sprague-Dawley rats were orally pretreated with ethanol (50 percent in water) in a single dose, on the basis of 5 g/kg body weight. Sixteen to eighteen hours after pretreatment, each group (6 rats) was exposed to TCI in a dynamic exposure chamber. Exposure levels were 100, 2000, 5000, and 10,000 ppm (538, 10,760, 26,900, and 53,800 mg/m^3) TCI (3 rats in the highest exposure category died). Animals ingesting ethanol prior to exposure to TCI showed a statistically significant ($p<0.05$) increase in serum SGOT, SGPT, and serum isocitric dehydrogenase (SICD) at 5000 ppm (26,900 mg/m^3). After exposure to 5000 ppm (26,900 mg/m^3) for 4 h, SGOT of animals pretreated with ethanol was about 20 times the control level, whereas exposed rats not receiving ethanol had normal SGOT values. Animals exposed to 2000 ppm (10,760 mg/m^3) or less for 4 h showed no marked alteration of serum enzyme levels. Neither ethanol-treated nor nontreated rats showed any alterations of serum enzyme levels when exposed for 8 h to 100 ppm. All rats exposed to 5000 ppm (26,900 mg/m^3) TCI showed slight to widespread degenerative lipid infiltration of the liver. Ethanol-treated rats (before TCI exposure) exhibited early centrilobular necrosis with slight acute inflammatory cellular reaction.

Muller et al. (1975) proposed that TCI and ethanol undergo similar metabolic steps, thus allowing for a variety of interactions, depending on dose levels and the sequence of exposure. When simultaneous exposure occurs, TCI and ethanol may compete for both alcohol dehydrogenase and microsomal mixed-function oxidase. Intolerance of TCI-exposed workers to alcohol has been described by Defalque (1961). It is often manifested as red flushes on the face and upper parts of the body and has been referred to as "degreasers flush." Stewart et al. (1974) observed this effect in seven individuals given as little as 0.5 ml ethanol/kg during exposure to 20,100, and 200 ppm (108, 538, 1076 mg/m^3) TCI for 1, 3, or 7.5 h. Alcohol intolerance among TCI-exposed workers has also been described by Bardodej et al. (1952) and Bardodej and Vyskocil (1956).

Traiger and Plaa (1974) reported that isopropyl alcohol or acetone, administered to mice in an 18-h pretreatment, potentiated the hepatotoxic response to 2 doses of TCI, 11.1 mmole/kg and 16.7 mmole/kg.

Cornish et al. (1977) found that rats given i.p. doses of 0.5, 1.0, and 2.0 ml TCI/kg showed progressively increasing levels of SGOT. Pretreatment with either phenobarbital or ethanol or both had no effect on this response. This lack of response with ethanol is in contrast to the earlier study by Cornish and Adefuin (1966) but may reflect different routes of exposure.

Utesch et al. (1981), in developing their animal model for solvent abuse (see 5.4.2), found that pretreatment of Wistar-Munich rats with ethanol exacerbated the CNS-depressant effects of high doses of TCI. A group of rats was given 5 ml/kg ethanol orally 1 h before initiation of the TCI inhalation regimen: 15 exposures cycles in a 5-h period; each cycle consisted of 5 min of inhalation of 15,000 ppm TCI followed by a 15-min solvent-free interval. Ethanol was shown to cause significant prolongation of the animals' ability to regain the righting reflex after the second and each subsequent TCI exposure. Administration of ethanol alone did not result in an increase in levels of SGOT or SGPT in histopathologic alterations of the liver or kidneys. No elevations in SGPT were seen in any of the groups that received both alcohol and TCI. Histopathologic examination of the liver and kidneys revealed no evidence of chemically-induced injury in any animals. Fasting for 16 h before the 5-h intermittent TCI exposure regimen had no apparent effect on the combined CNS-depressant action of ethanol and TCI. No histopathological effects were observed in the livers or kidneys of these animals.

Moslen et al. (1977) also reported that fasting had little effect on SGOT and SGPT levels in rats which inhaled 10,000 ppm TCI continuously for 2 h. However, pretreatment of rats with phenobarbital in combination with fasting produced significant elevations in the levels of these serum enzymes.

Cornish et al. (1973) reported that phenobarbital pretreatment produced no additional effect on SGOT above that produced by 1.0 and 2.0 ml TCI/kg, given i.p. to Sprague-Dawley rats. Lower doses of TCI, 0.5 and 0.3 ml/kg, had no effect on SGOT.

Pretreatment of male albino rats with either phenobarbital or 3-methyl cholanthrene was shown by Carlson (1974) to potentiate the hepatotoxicity of TCI. Rats were injected with either 50 mg phenobarbital/kg i.p. for 4 days or 3-methyl cholanthrene in corn oil, 40 mg/kg i.p. for 2 days. Animals were exposed to TCI 24 h after the last dose of phenobarbital or 48 h after the last dose of 3-methyl cholanthrene in a dynamic inhalation chamber. Rats were sacrificed 22 h after exposure. Phenobarbital pretreatment increased SGOT and SGPT three-fold above TCI controls. TCI exposure was 10,400 ppm (55,952 mg/m^3) for 2 h. Similarly, 3-methyl cholanthrene increased SGOT and SGPT; 3-methyl cholanthrene pretreatment also increased the level of SICD about twenty-fold, compared to TCI controls.

5.8 SUMMARY OF TOXICOLOGICAL EFFECTS

There is no reliable information concerning the toxicological effects in humans of chronic exposure to levels of TCI below the TLV (100 ppm). Based upon acute human overexposure information and limited animal testing, it is unlikely that chronic exposure to TCI at levels found or expected in ambient air would result in liver or kidney damage. Such damage has not been generally found even when exposure greatly exceeds the TLV.

The first sign likely to be observed upon exposure to TCI is central nervous system (CNS) dysfunction. In limited, acute controlled human exposure situations, alterations in task performance have been reported only at levels in excess of 100 ppm. There have been few in-depth studies, in rodent species, of the effects of TCI on the nervous system and behavior. Most endpoints that have been evaluated were studied in only a preliminary manner.

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6. TERATOGENICITY, EMBRYOTOXICITY, AND REPRODUCTIVE EFFECTS

Because of its widespread use, TCI has been studied for teratogenic potential. Teratology studies have been performed in rats, mice, and rabbits using doses of TCI which, in some studies, produced slight signs of maternal toxicity. Also, TCI is known to be metabolized in the maternal host by hepatic metabolizing enzymes (and possibly also in the fetal liver) to chloral hydrate and then to trichloroethanol and trichloroacetic acid. These metabolites, particularly trichloroethanol, have also been shown to readily cross the human placenta into the fetal circulation and amniotic fluid (Bernstine and Meyer, 1953; Bernstine et al., 1954) and also into the breast milk of nursing mothers (Bernstine et al., 1956). There is little information available on the fetotoxic and teratogenic potential of these metabolites. Chloral hydrate and its metabolite, trichloroethanol, have been in common medical use for a great many years as hypnotics, including use during pregnancy, but with no reported adverse teratogenic, fetotoxic, or reproductive effects (Goodman and Gilman, 1980). Trichloroethanol has been administered to three animal species at various stages of pregnancy, at levels as high as 700 mg/kg/day, without dose-related effects (Physicians Desk Reference, 1981). Other studies in avian embryo systems (Fink, 1968; Elovaara et al., 1979) have indicated that TCI disrupts embryogenesis in a dose-related manner. However, because administration of TCI directly into the air space of chicken embryo is not comparable to administration of dose to animals with a placenta, it is not possible to interpret this result in relationship to the potential of TCI to cause adverse effects in animals or humans. The following discussion of studies subscribes to the basic viewpoints and definitions of the terms "teratogenic" and "fetotoxic" as summarized and stated by the U.S. Environmental Protection Agency (1980):

> Generally, the term "teratogenic" is defined as the tendency to produce physical and/or functional defects in offspring _in utero_. The term "fetotoxic" has traditionally been used to describe a wide variety of embryonic and/or fetal divergences from the normal which cannot be classified as gross terata (birth defects) -- or which are of unknown or doubtful significance. Types of effects which fall under the very broad category of fetotoxic effects are death, reductions in fetal weight, enlarged renal pelvis edema, and increased incidence of supernumerary ribs. It should be emphasized, however, that the phenomena of terata and fetal toxicity as currently defined are not separable into precise categories. Rather, the spectrum of adverse embryonic/fetal effects is continuous, and all deviations from the normal must be considered as examples of developmental toxicity. Gross morphological terata represent

but one aspect of this spectrum, and while the significance of such structural changes is more readily evaluated, such effects are not necessarily more serious than certain effects which are ordinarily classified as fetotoxic--fetal death being the most obvious example.

In view of the spectrum of effects at issue, the Agency suggests that it might be useful to consider developmental toxicity in terms of three basic subcategories. The first subcategory would be embryo or fetal lethality. This is, of course, an irreversible effect and may occur with or without the occurrence of gross terata. The second subcategory would be teratogenesis and would encompass those changes (structural and/or functional) which are induced prenatally, and which are irreversible. Teratogenesis includes structural defects apparent in the fetus, functional deficits which may become apparent only after birth, and any other long-term effects (such as carcinogenicity) which are attributable to in utero exposure. The third category would be embryo or fetal toxicity as comprised of those effects which are potentially reversible. This subcategory would therefore include such effects as weight reductions, reduction in the degree of skeletal ossification, and delays in organ maturation.

Two major problems with a definitional scheme of this nature must be pointed out, however. The first is that the reversibility of any phenomenon is extremely difficult to prove. An organ such as the kidney, for example, may be delayed in development and then appear to "catch up." Unless a series of specific kidney function tests are performed on the neonate, however, no conclusion may be drawn concerning permanent organ function changes. This same uncertainty as to possible long-lasting aftereffects from developmental deviations is true for all examples of fetotoxicity. The second problem is that the reversible nature of an embryonic/ fetal effect in one species might, under a given agent, react in another species in a more serious and irreversible manner.

6.1 ANIMAL STUDIES

6.1.1 Mouse

Schwetz and his associates (Schwetz et al., 1975; Leong et al., 1975) investigated the potential of TCI to adversely affect fetal development in Swiss-Webster mice (Table 6-1). Twelve mice were exposed by inhalation to TCI (99.24-percent pure) at an air concentration of 300 ppm (1614 mg/m^3) for 7 h daily on days 6 through 15 of gestation. Day 0 of gestation was designated for the appearance of a vaginal plug. Concurrent pregnant control mice (30) were exposed to filtered air. The mice were observed daily throughout pregnancy, and maternal body weights were recorded on days 6, 10, 16 and 18 of gestation as an index of maternal toxicity. Following caesarean section on day 18, the fetuses were weighed, measured, (crown-rump length), sexed, and then examined for external anomalies. One-half of the fetuses were examined for soft tissue malformation, using free-hand sectioning, and one-half of the fetuses were

TABLE 6-1. SUMMARY OF ANIMAL STUDIES OF FETOTOXIC AND TERATOGENIC POTENTIAL OF TCI

	TCI purity	Species	Conditions (mode of administration, dosage, and duration of exposure)	Measures	Results
Fink (1968)	Unknown	Chick embryo	Vapor exposure, 10,000 ppm	Mortality anomalies	Increase Slight increase
Elovaara (1979)	Reagent grade	Chick embryo	Injected; 5 - 100 µmole per egg in 25 µl olive oil	LD_{50}	50 - 100 µmole/egg (16 % malformations in total survivors)
Schwetz et al. (1975)	Technical 99.24 % TCI 0.76 % stabilizers and impurities (Neu-tri)	S - D Rat ♀ S - W mouse ♂	Inhalation; 300 ppm, 7h/d on 6 - 15-d gestation	Embryo toxicity, teratogenicity (maternal toxicity)	+ - (+)
Bell (1977)	Technical (Trichlor 132)	CR - SD rat ♀	Inhalation; 300 ppm, 6h/d on 6 - 15-d gestation	Embryo toxicity, teratogenicity (maternal toxicity)	+ - (+)
Dorfmueller et al. (1979)	Technical 99 %+ TCI 0.2 % epichlorohydrin (Neu-tri)	Rat ♀ (Long-Evans)	Inhalation, 1800 ppm a) Premating: 6h/d, 5d/wk for 2 wk b) Premating for 2 wk + first 20-d gestation, daily c) First 20-d gestation, daily	Embryo toxicity, teratogenicity, offspring behavioral evaluation (maternal toxicity)	+ - (+)
Beliles et al. (1980)	Technical 99.9 %	CR - SD Rat ♀ Rabbit ♀	Inhalation; 500 ppm, 7h/d, 5d/wk Premating 3 wk + first 18-d gestation, daily (rat) + first 21-d gestation, daily (rabbit)	Embryo toxicity, teratogenicity (maternal toxicity)	+ - (+)

cleaned, stained, and examined for skeletal malformations. One fetus in each litter was subjected to microscopic examination.

Exposure to TCI had no effect on either the mean, absolute, or relative weight of the liver of mice dams when measured at the time of caesarean section. Exposure had no effect on the average number of implantation sites per litter, litter size, the incidence of fetal resorptions, fetal sex ratios, or fetal body measurements. The incidence of gross anomalies observed by external examination was not significantly greater than among control litters. TCI exposure had no effect on the incidence of skeletal anomalies, and microscopic examination revealed no abnormalities of organs, tissues, or cells. These investigators concluded that 300 ppm (1614 mg/m^3) TCI caused no significant maternal, embryonal or fetal toxicity, and that TCI was not teratogenic in Swiss-Webster mice at this dose level.

6.1.2 Rat

Schwetz et al. (1975) carried out a study in Sprague-Dawley rats. The design of the study was similar to their protocol for mice described above. TCI (300 ppm; 1614 mg/m^3) was administered by inhalation, 7 h daily, to 18 pregnant rats during organogenesis, the period when the greatest susceptibility to teratogenic effect is present (days 6 to 15 of gestation). Controls (30) were provided filtered air. Day 0 of gestation was defined as the day spermatozoa were observed in vaginal smears. The animals were killed on day 21 of gestation, caesarean sections were performed, and fetuses in each litter were examined for external malformations. One-half of the fetuses in each litter were then examined for soft tissue malformations, and the other half examined for skeletal malformations. One fetus from each litter was randomly selected for serial sectioning and histological evaluation. Schwetz et al. (1975) reported a small but statistically significant inhibition of maternal body weight gain when dams were exposed to 300 ppm (1614 mg/m^3) TCI. However, this dose had no effect on either the mean, absolute, or relative weight of the maternal host livers when measured at the time of caesarean section. Exposure to TCI had no effect on the average number of implantation sites per litter, litter size, the incidence of fetal resorptions, fetal sex ratios, or fetal body measurements. No increased incidence of soft tissue or skeletal anomalies was observed, and histopathological examination revealed no abnormalities of organs, tissues, or cells. Schwetz et al. (1975) concluded that 300 ppm (1614 mg/m^3) TCI caused no significant maternal, embryonal or fetal toxicity in Sprague-Dawley rats.

Bell (1977) reported a similar study, conducted by Industrial Bio-Test Lab. Inc. (Northbrook, Ill.), on the teratogenic and fetal toxic effects of TCI on Charles River rats. TCI (300 ppm; 1614 mg/m^3) was administered to 17 dams by inhalation exposure, 6 h per day, from day 6 through day 15 of gestation. Control pregnant animals (21) were killed on day 20 of gestation. Caesarean sections were performed, fetal swellings and implantation sites counted, and resorption sites and number of corpora lutea determined. Viable fetuses were counted, and all fetuses were removed and weighed. External examination of the fetuses was made for gross anomalies. Two-thirds of the fetuses from each litter were examined for skeletal development, and the remaining one-third of the fetuses were evaluated for soft tissue abnormalities. The investigators found that 300 ppm (1614 mg/m^3) TCI exposure produced no evidence of maternal toxicity, although a small but significant reduction of mean weight gain by gestation day 15, in comparison to control dams, was recorded. There were no mortalities or adverse behavioral reactions, gross fetal anomalies, or differences in mean weights of fetuses exposed to TCI in comparison to unexposed controls. The numbers of corpora lutea, implantation sites, resorption sites, and fetuses were similar among exposed and control dams. No evidence for teratogenicity was observed in fetuses from either exposed or unexposed dams.

Beliles et al. (1980) reported on a study of teratogenic and fetotoxic potential of TCI in Sprague-Dawley rats conducted by Litton Bionetics, Inc. (Kensington, MD.). The experimental design of the study included pregestational as well as gestational exposure (Table 6-2).

TABLE 6-2. EXPERIMENTAL DESIGN OF BELILES et al. STUDY (1980) ON SPRAGUE-DAWLEY RATS

Group	No.	TCI ppm	Days of Exposure (Controls exposed to air only) Pre-gestational	Gestational
1	21	0 (control)	None	0-18
2	16	0 (control)	(5d/wk) 3 wk	0-18
3	20	500	None	0-18
4	16	500	(5d/wk) 3 wk	0-18
5	20	500	None	6-18
6	32	500	(5d/wk) 3 wk	6-18

Exposure to TCI, 500 ppm (2690 mg/m^3) in air, was 7 h/d, 5 d/wk, during a 3-wk pre-gestational period, and 7 h/d each day for days 0 to 18 and days 6 to 18 of gestation. Positive identification of spermatozoa in the vaginal canal was taken as evidence of mating and designated as day 0 of gestation. The dams were killed on day 20 of gestation, caesarean section was performed, visceral and thoracic organs were examined, and the numbers corpora lutea per dam determined. The maternal livers, kidneys and lungs were weighed. The numbers of implantation sites, live and dead fetuses, and resorption sites were recorded. The fetuses were examined for gross external anomalies and weighed, and crown-rump length and sex were determined. One-half of the fetuses of each litter were sectioned and examined for changes in the soft tissues; the remaining fetuses were examined for skeletal abnormalities.

The findings reported in this study indicate that maternal toxicity was not a factor in data interpretation; means of body weight, liver, kidneys, and lungs of test dams were within normal expected variation of control dams. No deaths occurred in the exposed groups. The investigators concluded that neither the frequency nor character of the macro- or microscopic findings in the treated groups indicated an adverse effect on fetal growth and development, or a teratogenic potential for TCI at the dose level of 500 ppm.

Dorfmueller et al. (1979) conducted a study of the teratogenic and fetotoxic effects of TCI in Long-Evans rats exposed to a inhalation concentration of 1800 ppm (9684 mg/m^3) in air. Their experimental design was similar to that of Beliles et al. (1980) insofar as the animals were exposed during the pre-gestational period in addition to during gestation (Table 6-3).

The premating exposure was conducted for 6 h/d, 5 d/wk for 2 wk, and the gestational exposure was 6 h/d each day for days 0 to 20. Day 1 of pregnancy was considered to be the day on which sperm from mating were observed in vaginal smears from mating. On day 21 of gestation, 15 out of 30 dams/treatment group were sacrificed; maternal livers were removed, weighed, and frozen for later enzyme analysis. Caesarean sections were performed and the number of live, dead, and resorbed fetuses counted. Each fetus was weighed, the sex was recorded, and then examined for external anomalies. Four fetuses from each litter were examined for soft tissue changes, and four fetuses were evaluated for skeletal abnormalities. Livers were removed from the remaining fetuses for measurement of mixed-function oxidase activity.

TABLE 6-3. EXPERIMENTAL DESIGN OF DORFMUELLER et al. STUDY (1979) ON LONG-EVANS RATS

Group	No.	TCI (ppm)	Days of Exposure	
			Pre-gestational	Gestational
A	30	1800 ± 200	5d/wk; 2 wk	1 - 20
B	30	1800 ± 200	5d/ wk; 2 wk	none
C	30	1800 ± 200	none	1 - 20
D	30	none	none	none

Postnatal behavioral evaluation was conducted on the offspring of the remaining 15 dams/treatment group which were allowed to litter. At weaning, 2 male and 2 female pups from each litter were randomly selected for observation of activity measurements at 10 days of age (Motility Meter) and 20 and 100 days of age (Activity Cage).

Dorfmueller et al. (1979) found that the female rats exposed to 1800 ppm (9684 mg/m^3) TCI before mating and/or during pregnancy did not exhibit any signs of maternal toxicity. Weight gains throughout pregnancy were normal in relation to filtered air controls, and the relative and absolute maternal liver weights indicated that no significant treatment effects occurred. There was no indication of embryotoxicity; no significant treatment effects or interactions were found in numbers of corpora lutea or implantation sites/ litter, fetal body weights, resorbed fetuses/litter, or sex ratios. No significant treatment effects were observed in the analysis of total soft tissue anomalies. However, the incidence of total skeletal anomalies was increased in group C because of an increased incidence of incomplete ossification of the sternum, but when the frequency of this particular anomaly alone was compared in group C versus group D, the investigators found no significant difference. Incomplete ossification of the sternum is thought to be indicative of delays in the general process of skeletal ossification which are not necessarily irreversible. Thus, prenatal exposure to 1800 ppm (9684 mg/m^3) TCI caused an elevation of minor anomalies indicative of development delays in maturation, but not of major malformations. Also, the study indicated no treatment effect on the general activity of the offspring at 10, 20, and 100 days of age. There was, however, a small but statistically significant depression in postnatal weight gains of offspring in the premating exposure groups (A and B), the biological significance of which was not apparent. The offspring are being maintained until 18 months of age to further assess postnatal behavior and transplacental carcinogenicity potential. This study revealed no results indicative of treatment-related maternal toxicity, embryotoxicity, teratogenicity, or significant postnatal behavioral deficits from exposure, pre-gestational and/or gestational, to 1800 ppm (9684 mg/m^3) TCI in air.

6.1.3 Rabbit

Beliles et al (1980) also studied the fetotoxicity and teratogenicity of TCI in the female New Zealand white rabbit. Their experimental design is presented in Table 6-4.

TABLE 6-4. EXPERIMENTAL DESIGN OF BELILES et al. STUDY (1980) ON NEW ZEALAND WHITE RABBITS

Group	Animals/ group	TCI ppm	Days of Exposure Pre-gestational	Gestational
1	21	0 (control)	None	0-21
2	18	0 (control)	5d/wk; 3 wk	0-21
3	23	500	None	0-21
4	19	500	5d/wk; 3 wk	0-21
5	23	500	None	7-21
6	25	500	5d/wk; 3 wk	7-21

Exposure to TCI, 500 ppm (2690 mg/m^3) in air, was 7 h/d, 5 d/wk during a 3-wk pre-gestational period, and/or daily for days 0 to 21 or days 7 to 21 of gestation. Observed copulation and/or the presence of sperm in the vaginal canal established day 0 of gestation. On day 30 of gestation, the dams were killed, caesarean-sectioned, and the corpora lutea per dam determined. The maternal livers, kidneys, and lungs were removed, weighed, and examined; the remaining visceral and thoracic organs were also examined. The number of uterine implantation sites, live and dead fetuses, and resorption sites were recorded. The fetuses were examined for gross external anomalies and weighed, and then crown-rump length and sex ratios were determined. One-half of the fetuses of each litter were sectioned and examined for changes in the soft tissues; the remaining fetuses were examined for skeletal abnormalities.

The number of deaths in the TCI-treated groups was not significantly greater than among controls. Deaths occurring during the experiment account for the unequal numbers of animals in the groups. The mean body weights during pregestational and gestational periods were determined, and there were no remarkable differences between control and treated rabbits.

This study provided no evidence of maternal toxicity from 500 ppm (2690 mg/m^3) exposure to TCI. Means of body weight, liver, and lungs were within normal expected variation of control dams. A small increase in means of kidney weights for groups 2, 4, and 6 (compared to group 1) was found, but these increases were judged to be unrelated to treatment. No difference was observed in the mean weight of fetuses exposed to TCI in comparison to those of unexposed controls. The numbers of corpora lutea, implantation sites, resorption sites, fetuses and sex ratio were similar among exposed and control dams.

Gross examination of fetuses, with regard to external morphology and internal abnormalities, revealed no unusual changes except for hydrocephalus in 4 fetuses of 2 litters of group 3. This anomaly occurred only in the one treatment group 3 and the malformation was not observed in the litters of rabbits that were exposed to 500 ppm (2690 mg/m^3) TCI during a 3-week pre-gestational period plus the same gestational period (Group 4). The authors confirmed the anomaly as an external hydrocephalus, which, in their experience, does not occur in untreated control rabbits. They, therefore, felt that, although not statistically significant, this finding could not be discounted entirely as occurring by chance. Unless other factors can be identified which are responsible for increasing the susceptibility of the rabbits during the gestational period, it would be expected that the malformation would occur in animals exposed to the same dose of TCI for a longer period.

Examination of the fetuses for soft tissue anomalies showed no changes (except external hydrocephalus discussed above) that were not frequently observed in 30-day-old rabbit fetuses of the strain and source used in the study. Skeletal examination revealed no unusual features, except for an increased incidence of changes related to retarded bone ossification, which were not considered malformations as such. Neither frequency nor the character of these changes in the treated groups indicated an adverse effect on fetal growth and development or of a teratogenic potential. In summary, this study in rabbits exposed to 500 ppm (2690 mg/m^3) TCI revealed no evidence of maternal toxicity or embryotoxicity; the authors did report the occurrence of hydrocephalus in a few fetuses of one of the study groups, but no definitive conclusion was made regarding this occurrence.

6.2 FETOTOXICITY IN HUMANS

There are no epidemiologic studies in the literature specifically investigating fetotoxicity or overt birth anomalies that may occur from TCI exposure in the workplace.

Corbett and his associates (1972; 1974) reported an increased incidence of miscarriages in operating room nurses exposed to various general anesthetics (volatile halogenated aliphatic hydrocarbons). TCI was only one of several anesthetics in use, and, therefore, it is impossible to make a specific association of TCI with the miscarriages. In addition, the study and its methodology have been severely criticized by Cote (1975), particularly with respect to clinical definition of anomalies and to methods of clinical evaluation.

Aviado et al. (1976), in his review, concluded that TCI exposure may produce respiratory difficulties in neonates. However, respiratory effects on neonates have not been established, and TCI has been in use for many years in Europe as a self-administered inhalant analgesic and anesthetic during labor without reported effects.

6.3 SUMMARY

Well-designed inhalation exposure studies in the mouse (300 ppm), and rat (300,500 and 1800 ppm; 1614, 2690, and 9684 mg/m^3) by different investigators demonstrate no significant maternal toxicity, embryo toxicity, teratogenicity, or postnatal behavioral effects. In rabbits, 500 ppm (2690 mg/m^3) inhalation exposure provides no evidence of maternal toxicity or embryo toxicity, but an assessment of teratogenic potential was made based on only a few hydrocephalic fetuses observed in one group (of four) exposed to TCI.

At present, the available studies do not indicate that TCI is toxic to the fetus at levels below the maternally toxic level. Also, no definitive clinical evidence of fetotoxicity or teratogenicity from TCI exposure has been reported. Therefore, the available information does not indicate that the conceptus is uniquely susceptible to the effects of TCI. It should be noted, however, that additional studies of appropriate rodent species are needed to more fully examine the teratogenic potential of TCI.

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7. MUTAGENICITY

Trichloroethylene (TCI) has been tested for its ability to cause gene mutations in bacteria, yeast, higher plants, insects, and rodents. It has also been evaluated for its ability to cause chromosome aberrations in insects, rodents, occupationally exposed workers and in cultured cells, and for its ability to cause other effects which are indicative of DNA damage (gene conversion, mitotic recombination, sister chromatid exchange and unscheduled DNA synthesis and DNA alkylation). The results of these tests will be discussed below and summarized in Tables 7-1 to 7-9. Consideration will also be given to studies bearing on the potential of TCI to reach the germinal tissue of mammals.

7.1 GENE MUTATION STUDIES

7.1.1 Prokaryotic Test Systems (Bacteria)

Eight reports were evaluated concerning testing TCI for its mutagenic potential in bacteria. Seven used *Salmonella typhimurium* (Henschler et al. 1977, Simmon et al. 1977, Margard 1978, Waskell 1978, Baden et al. 1979, Bartsch et al. 1979, Kline et al. 1982), and one employed *Escherichia coli* (Greim et al. 1975).

Because of the possibility of mutagenic contaminants in TCI samples, Henschler et al. (1977) chemically analyzed and subsequently tested samples of TCI for mutagenicity. They determined that a sample of technical grade TCI used in the National Cancer Institute carcinogenicity bioassay (NCI 1976) contained the following impurities (%w/w) by gas chromatography-mass spectrometry analysis: epichlorohydrin, 0.22; 1,2-epoxybutane, 0.20; carbon tetrachloride, 0.05; chloroform, 0.01; 1,1,1-trichloroethane, 0.035; diisobutylene, 0.02; ethylacetate, 0.052; pentanol-2, 0.015; butanol, 0.051. These impurities, were tested for mutagenicity with and without S9 mix from PCB-induced Wistar rat livers. In Salmonella typhimurium, two of them, epichlorohydrin and 1,2-epoxybutane, were found to be mutagenic in tests conducted without activation. Increases of 25-fold and 8-fold over the spontaneous revertant rates were observed for the two compounds, respectively (extrapolated from Figure 1 of the paper). Negative responses were observed when the tests were repeated using S9 mix. Negative responses were also obtained with carbon tetrachloride, chloroform, and 1,1,1-trichloroethane both with and without activation, but no data were presented. Similarly, no increased mutation frequency was obtained with "purified" TCI (source and purity not given). Roughly 140 TA100 revertants were observed at each point tested over the entire dose range (from 1.12 x 10^{-3}M to 1.12 M) both with and without metabolic activation; the spontaneous revertant count was 138 ± 8 (without activation) and 242 ± 10 (with activation) colonies per plate. In their testing, Henschler et al. (1977) added the chemical substances directly to the top agar. No precautions were reported to have been taken to prevent excessive evaporation of the test compound, and thus, the significance of the negative response obtained with TCI is weakened by the possibility that the material may have evaporated before adequately exposing the indicator cells. This aspect of the study design could not be evaluated because no toxicity data were presented. The positive response after exposure to other volatiles like 1,2-epoxybutane may reflect the potency of these materials compared to TCI. Based on their results, the authors concluded that the carcinogenic activity of technical grade TCI in the NCI bioassay experiment was probably predominantly, if not exclusively, due to the epoxide contaminants. This study is inadequate for reaching these conclusions for a number of reasons including flaws in the study design such as those mentioned above (i.e., the lack of a positive control requiring metabolic activation and not taking precautions to prevent the escape of TCI).

Margard (1978) also conducted studies addressing the hypothesis that the mutagenic activity of technical grade samples of TCI is due to mutagenic stabilizers and not TCI itself by comparing the mutagenic properties of technical and purified grades of TCI in the Salmonella typhimurium/mammalian microsome plate incorporation assay. He used tester strains TA1535, TA1537, TA1538, TA98, and TA100 in the presence and absence of a metabolic activation system (S9 mix) from adult Sprague-Dawley rats induced with Aroclor-1254. Prior to plating, two aliquots of cells were taken. One was diluted and grown on complete media for determining cell survival and the other was grown on histidine deficient plates for selection of his^+ revertants. Cells were then incubated for 48 hours at 37°C and colony counts were made. The technical grade contained 0.08% (w/w) epichlorohydrin, 0.02% N-methylpyrrole, 0.13% N-propyl alcohol, 0.05% nitromethane, 0.10% 1,1,1-trichloroethane, and 0.45% butylene oxide. The purified grade contained no detectable epoxides nor other stabilizing ingredients (written communication with L. Schlossberg, Detrex Chemical Industries, Inc.). A concentration-related increase in the number of revertants was observed, as described in Table 7-1, in strain TA1535 both with and without metabolic activation after exposure to technical grade TCI. This sample was not mutagenic in strains TA98, TA100, TA1537, and TA1538. Purified TCI was not mutagenic in any strains. TCI levels $\geq$ 0.1 ml/plate were toxic as indicated by reduced cell viability (49-86% killing). It has been reported that precautions were taken to inhibit evaporation of the test samples during treatment of the cells (Schlossberg, personal communication), but it was not specified in writing what those precautions were. The toxicity data suggest that the cells were exposed to the test material, but it is not possible to determine toxicity

TABLE 7-1. MUTAGENICTY OF TECHNICAL GRADE TRICHLOROETHYLENE IN SALMONELLA/MAMMALIAN MICROSOME ASSAY (adapted from Margard 1978)

		Activation			Nonactivation		
		Toxicity		Mutagenicity	Toxicity		Mutagenicity
Tester Strain	Conc. ml/plate	Plate Count	Rel. Via.*	No. Rev.†	Plate Count	Rel. Via.*	No. Rev.†
TA1535	1.0						
	0.5						
	0.1	27	0.14	58	22	0.14	58
	0.05	142	0.72	40	122	0.78	42
	0.01	185	0.94	17	149	0.95	21
	0	196	--	24	157	--	23
TA100	1.0						
	0.5						
	0.1	53	0.19	184	66	0.28	173
	0.05	207	0.73	263	167	0.71	181
	0.01	255	0.90	123	232	1.0	136
	0	283	--	167	234	--	160
MUTAGENICITY OF PURIFIED GRADE TRICHLOROETHYLENE							
TA1535	0.1						
	0.5						
	0.1	97	0.39	17	89	0.51	15
	0.05	172	0.70	19	121	0.69	20
	0.01	22	0.90	21	156	0.89	20
	0	246	--	22	175	--	19
TA100	1.0						
	0.5						
	0.1	175	0.55	70	166	0.54	114
	0.05	175	0.55	142	162	0.53	158
	0.01	225	0.71	157	354	0.82	145
	0	317	--	154	305	--	158

*Relative viability of tests compared to the control plates.
†Number of revertants observed (average).

adequately in a plate incorporation test. To do this a liquid suspension assay must be done. Appropriate positive controls were reported and demonstrated the S9 had metabolic activity. From the data presented, it is not clear how many plates were employed for each dose nor is it clear whether or not replicate testing was performed. Under the conditions of test "purified" TCI was not mutagenic.

To overcome the concern that the volatility of certain halogenated hydrocarbons may prevent them from being adequately tested in standard plate incorporation tests, Waskell (1978) investigated the mutagenic properties of commonly used volatile anesthetics, including TCI, and several of their known metabolites in testing conducted in sealed chambers. Salmonella typhimurium strains TA100 and TA98 were used in tests for gene reversion. Anesthetic-grade TCI from Ayerst was tested in closed containers at doses up to 10% in the atmosphere. The purity of the sample tested was not reported, but it would have been high with an approximate minimum purity of 99.5% and stabilization by 0.1% thymol (personal communication with Ayerst). Holes were punched in the top of the Petri dishes to allow the test vapor access to the bacterial strains. Tests were conducted in the presence and absence of phenobarbital- and PCB-induced Sprague-Dawley rat liver microsomes. No toxicity testing was conducted nor were the actual concentrations of TCI measured in the exposure chamber so it is not known whether the bacteria received a sufficiently high dose. After a 48-hour exposure TCI did not produce a mutagenic effect in strains TA100 and TA98 with or without metabolic activation. However, no data were presented to support this conclusion. With respect to the metabolites of TCI, the non-volatile compound 1,1,1-trichloroacetic acid ($\geq$ 99.0% pure, obtained from Matheson, Coleman, and Bell, personal communication with Matheson, Coleman, and Bell), chloral hydrate (recrystallized from chloroform six times to achieve a purity of $\geq$ 99.0%), and trichloroethanol (purity not determined, personal communication with L. Waskell, V.A. Hospital, San Francisco, CA) were tested at maximum nontoxic quantities in plate incorporation tests. Doses of trichloroethanol at 7.5 mg/plate and trichloroacetic acid at 0.45 mg/plate were not mutagenic in strains TA98 and TA100 chloral hydrate at 10 mg/plate increased the number of TA100 revertants above control (200) by 121 with metabolic activation and 89 without metabolic activation. The data are inconclusive but suggestive of a weak positive response. These strains were responsive to the positive control chemicals diethyl sulfate and 2-aminofluorene.

Further testing was done to evaluate the dose-response of chloral hydrate-induced mutagenesis in strain TA100. The number of revertants per plate in the control groups were 190 and 210 with and without metabolic activation, respectively. Doses of chloral hydrate from 0.5 mg/plate to 10.0 mg/plate did not result in a mutation frequency greater than twofold above the spontaneous level. However, increases in the number of revertants per plate above control values were observed both with and without metabolic activation: 255 to 260 at 0.5 mg; 285 to 290 at 1.0 mg; 300 to 320 at 5.0 mg; and 290 to 310 at 10.0 mg. Although the author reported these increases to be statistically significant by the t test ($P < 0.01$), the increases are, at best, merely suggestive of a positive response. The negative response for the induction of gene mutations in bacteria induced by exposure to TCI reported by Waskell (1978) is consistent with the results of Henschler et al. (1977) and Margard (1978). However, equivocal weak positive responses suggestive of a positive effect have been reported by other investigators using air tight exposure chambers and metabolic activation.

In tests conducted employing Salmonella tester strain TA100 both with and without an exogenous S9 metabolic activation, Bartsch et al. (1979) assessed the mutagenic potential of over 20 chemical substances including a TCI sample obtained from Merck, Darmstadt, Federal Republic of Germany. The sample was reported to be 99.5% pure and contained no detectable amounts of epichlorohydrin or 1,2-epoxybutane at levels $\geq$ 1 ppm. Experiments were conducted using a vapor exposure assay in which Petri dishes containing a lawn of TA100 with or without S9 mix were placed, uncovered, in 9-liter desiccators for various lengths of time at 37°C in the dark. Upon completion of the exposure period the compound was removed, the plates were then covered, inverted, and placed in an incubator for an additional 48 hours at 37°C and scored. TCI was not found to be mutagenic under the conditions used (see below).

Concentration in Air (5 v/v)	Time (h)	Revertants/plate	
		+S9	-S9
Trichloroethylene			
0		75	70
8	16	85	72
20	16	55 (toxic)	50 (toxic)
Vinyl Bromide			
2	16	240	190
20	16	715	695

When the test conditions were modified such that plates containing a liver S9 fraction from phenobarbital-treated OF-1 mice, an NADPH-generating system, TA100, and 0.1 mM EDTA were preincubated at 37°C for 4 hours and then exposed to 5% TCI vapor in air for 2 hours, a nearly twofold increase over the spontaneous mutation frequency was observed (see below).

Concentration in Air (% v/v)	Time (h)	Revertants/plate	
		+S9	-S9
0		75	70
5*	2	100	80
5*	2†	135	70

*0.1 mM EDTA added to soft agar.

†Preincubated with S9, TA100, and 0.1 mM EDTA for 4 hours then exposed to TCI vapors.

The results of only one test were presented, but it was stated that near twofold increases over the spontaneous mutation frequency were observed consistently.

Baden et al. (1979) evaluated the mutagenic properties of TCI in *Salmonella typhimurium* strains TA100 and TA1535 both with and without S9 metabolic activation from PCB-induced male Sprague-Dawley rats. The sample was > 99.5% pure, contained no detectable epoxides, and was stabilized with 0.1% (w/w) thymol (personal communication with M. Kelly, V.A. Hospital, Palo Alto, CA). Liquid TCI was directly added to bacterial cell cultures for incubation at 37°C for 2 hours, and concentrations in the gas phase above the incubator, which were the same as those in the desiccator experiment described below, were monitored for the first 2 hours of exposure showing a variation of $\leq$ 10%. These cultures were plated and incubated for 2 days at 37°C before counting revertant colonies. TCI was not found to be mutagenic in this experiment. However, no monitoring was conducted after the initial 2 hours, and it is not known whether the desired exposure levels were maintained throughout the 2-day incubation period. Toxicity data were not reported and therefore it is not known whether the target cells were actually exposed to a sufficiently high concentration of the test material. In another experiment, reported in the same article, cultures were exposed to vapor concentrations ranging from 0.1 to 10.0% (± 10% tolerance) in a desiccator for 8 hours at 37°C with further incubation without treatment for 40 hours before counting of colonies. The experiment was repeated, and each test concentration was evaluated with triplicate assays. TCI vapor produced a less than twofold increase in the number of revertant colonies to approximately 30% above background (149 ± 12, n=15) at concentrations of 1% (196 ± 13, n=15) and 3% (194 ± 12, n=15) in TA100 with metabolic activation. No thymol control was reported, and the response was not dose-related. At best this study provides only suggestive, supportive evidence for mutagenic activity caused by TCI.

Simmon et al. (1977) also reported that reagent grade TCI, with no detectable epoxides, gave less than a twofold increase in revertant counts in Salmonella typhimurium strain TA100. The sample was 99.85% pure, and contaminants included 0.09% chloroform, 0.02% tetrachloroethylene, 0.01% pentachlorobutadiene, and 0.03% hexachlorobutene; epoxides were not detected (personal communication with R. Spanggord, SRI International). Plates containing TA100 with and without metabolic activation were exposed to TCI vapor evaporated from a known amount of liquid in a desiccator for 7 hours at 37°C before counting. TCI was detected as mutagenic only with metabolic activation. The number of revertants per plate was increased above the spontaneous control value of about 140 both with and without activation by TCI treatment as follows: 180, 230, 240, and 200 revertants at 0.5, 1.0, 1.5, and 2.0 ml TCI/9-liter desiccator, respectively, in the presence of S9 mix from B6C3F1 male mice; and 160, 210, 200, and 170 revertants at 0.5, 1.0, 1.5, and 2.0 ml TCI/9-liter desiccator, respectively, in the presence of S9 mix derived from PCB-induced male Sprague-Dawley rats. The revertant counts were extrapolated from Figure 22 of the paper. The results of these assays were reproduced several times (personal communication with V. Simmon, Genex Corp.), but no estimation of the variance of the results (stated to be low) was presented and no data are presented to allow its calculation.

Greim et al. (1975) exposed Escherichia coli strain K12 to 3.3 mM analytical grade TCI during 2 hours of incubation at 37°C in the presence of metabolic activation from phenobarbital-induced male NMRI mouse liver microsomes. A chemical analysis of the test sample was not done by the investigators (written communication with D. Henschler, University of Wurzburg); however, the approximate composition of the TCI product was probably: trichloroethylene, >99.9%; either $\leq$ 100 ppm diisopropylamine or $\leq$ 120 ppm of a combination of diisopropylamine, N-methylpyrrole, and 4-tertbutylphenyl as stabilizers; < 500 ppm of other chlorinated hydrocarbons combined (chloroform, carbon tetrachloride, 1,2-dichloroethane, 1,1,2-dichloroethylene [sic] , and tetrachloroethylene); no epoxides present at a detection level of 1 ppm (written communication with Dr. Fries and Dr. Koppe, E. Merck Company, the producer of the sample used in this study). Several loci in *E. coli* K12 were used to test for mutagenicity: back mutation at three loci, gal^+, arg^+, and nad^+, and the forward mutation MTR system which yields resistance to 5-methyl-DL-tryptophan. Survival of bacteria on complete medium at the TCI concentration used (3.3 mM) was 76 ± 4%. A weak twofold mutagenic effect of TCE, expressed as a percentage of the spontaneous mutation rate, was observed for arg^+, 232 ± 36, but the significance of this response cannot be ascertained because no increase was observed for the other markers; gal^+, 123 ± 23; MTR, 114 ± 18; nad^+, 100. The reporting of the conduct of testing and the observed results were inadequate. For instance, information concerning spontaneous mutation frequencies, number of replications, revertant count data, etc. for each test was not reported. Only one dose was used so it was not possible to obtain dose-response data. Thus, the significance of the results cannot be adequately assessed.

None of the tests described previously clearly showed TCI to be mutagenic. However, the weak, less than twofold, increases in revertant counts observed in several studies, using several samples of TCI and different strains and species of bacteria suggest that commercially available TCI may be weakly mutagenic to bacteria after metabolic activation. The data for concluding that TCI itself is mutagenic are best suggestive but the fact that weak but less than twofold responses were obtained with such samples only after metabolic activation argues against the possibility of the responses being caused by the presence of epoxide stabilizers because these agents are direct-acting mutagens.

It is important to note that only very weak positive responses (< 2-fold above the spontaneous level) were obtained with "purified" samples of TCI. In these studies the responses were not always dose-related and were observed only at very high doses (i.e. vapors ranging from 1 to 5% v/v). The data are not typical of mutagenic agents in Salmonella, but it is not possible to unequivocally rule out that TCI causing a slight increase in mutant yield. The studies described below discuss the observations from tests with other organisms.

7.1.2 Eukaryotic Test Systems

7.1.2.1 Plants--

7.1.2.1.1 Yeast--Employing the yeast Schizosaccharomyces pombe in a host-mediated assay with B6C3F1 mice, Loprieno et al. (1979) reported findings similar to those obtained in bacteria by Henschler et al. (1977). In this study the mutagenic potential of technical grade TCI (composition, % (w/w): trichloroethylene, 99; 1,2-epoxybutane, 0.19; epichlorohydrin, 0.09; ethylacetate, 0.04; n-methyl pyrrole, 0.02; diisobutylene, 0.03) was compared with that of a purified grade of TCI (composition w/w: trichloroethylene, 99.98%; chloroform, 10 ppm; carbon tetrachloride, 17 ppm; 1,1,2-trichloroethane, 21 ppm; 1,1,2,2-tetrachloroethane, 14 ppm; other contaminants, 16 ppm). One and one-half milliliters (2×10^9 cells/ml) of yeast cell suspension (SP ade 6-60/rad 10-198h$^-$) was injected into the peritoneal cavity of mice untreated (controls) or treated with a total volume of 0.5 ml doses of TCI (ranging from 0 to 2000 mg/kg) administered by gavage; cells were removed from the peritoneum and plated for counting of mutant colonies 6 or 16 hours after treatment. Each assay was performed in at least three animals. The report states that results of this test (summarized in Table 7-2) indicate that the technical grade material was weakly positive showing a dose-related increase in the induction of mutants. These results are judged to be equivocal for the following reasons. The linear regression analysis presented in the report was statistically incorrect, because it was calculated using mean values rather than the individual data points.

TABLE 7-2. MUTAGENICITY OF TRICHLOROETHYLENE AND EPICHLOROHYDRIN IN *S. POMBE* BY MEANS OF THE HOST-MEDIATED ASSAY IN B6C3F1 MICE

(16 hours of incubation)

(Loprieno et al. 1979)

Treatment	Dose mg/kg	Mutants M.F. x 10^{-4} (M ± S.E.)
Trichloroethylene technical grade	None (control)	0.94 ± 0.08*
	500	1.33 ± 0.28
	1,000	1.32 ± 0.29
	2,000	1.61 ± 0.45
Methyl methanesulfonate	50	4.16 ± 0.54
Trichloroethylene purified grade	None (control)	1.45 ± 0.06
	1,000	1.59 ± 0.22
	2,000	1.36 ± 0.21
Dimethylnitrosamine	100	16.73 ± 3.24
Epichlorohydrin	None (control)	1.66 ± 0.03
	2	1.50 ± 1.20
	10	2.05 ± 0.51
	50	3.71 ± 1.05

*Lower than the other negative control values.

The correlation coefficient was reported to be statistically significant ($r = 0.86$); recalculating using their data r was found to equal 0.35 which is not statistically significant. Furthermore, the number of mutants in the negative controls for the technical product was much less, by 44% and 35%, respectively, than those in the controls for the "purified" TCI and epichlorohydrin. The experimental values for the "purified" samples were in the same range as those for the technical material and they showed no increase in mutation frequency compared to the concurrent negative controls. The mutagenic response to epichlorohydrin tested by itself was, however, positive ($3.71 \pm 1.05 \times 10^{-4}$ mutants at 50 mg/kg) as was the response of the positive control dimethylnitrosamine ($16.73 \pm 3.24 \times 10^{-4}$ mutants at 100 mg/kg) indicating that for these chemicals the assay was working properly. No data were presented bearing on the ability of the administered TCI to reach the indicator organisms (e.g., chemical binding). The negative response in this host mediated assay obtained for the pure sample and the equivocal response for the technical sample in this study may be due to a number of reasons including the possibility that TCI (or its metabolites) did not reach the indicator organisms in the peritoneum under the test conditions employed.

Subsequent testing by the same laboratory (Rossi et al. 1983) was conducted to further investigate the mutagenic potential of pure and technical samples of TCI, and two stabilizers contained in technical grade sample: epichlorohydrin and 1,2-epoxybutane. *Schizosaccharomyces pombe* strain P1 was used in *in vitro* testing and in intraperitoneal and intrasanguineous host mediated assays. S9 mix from phenobarbital-or B-napthoflavone-induced mice or rat livers was used in the *in vitro* studies where TCI was tested at doses up to 100 mM. No increases in the frequency of forward mutations over negative controls (1.38 ± 0.67 revertants) was found. By contrast epichlorohydrin and 1,2-epoxybutane caused dose-related increases in mutant frequency up to about a 10-fold increase compared to negative controls at 6.4 mM and 12.8 mM, respectively.

Negative responses were obtained when 2 g/kg doses of TCI were used in intraperitoneal and intrasanguineous host-mediated assays with CD 1 x C57BL mice (Rossi et al. 1983). Negative responses were also obtained when epichlorohydrin and 1,2-epoxybutane were used in intraperitoneal host-mediated assays at doses up to 2g/kg. These data suggest that the intraperitoneal host mediated assay in mice with Schizosaccharomyces pombe may not be appropriate for testing halocarbons and their stabilizers.

Mondino (1979) also reported negative results for purified TCI in a yeast host-mediated assay. He used Schizosaccharomyces pombe and adult male B6C3F1 mice. The components of the TCI sample were identified as follows: trichloroethylene, 99.9%; water, evaporation residue, chloroform, 1,2-dichloroethane, carbon tetrachloride, 1,1,2-trichlorethane, other impurities, all $\leq$ 20 ppm; diisopropylamine, 198 ppm; diisobutylene, 104 ppm; butylhydroxytoluene, 12 ppm. Yeast cell suspension was injected into the peritoneum of the mice, who had received 500, 1000, or 2000 mg/kg dosages of trichloroethylene by gavage. Three animals comprised each treatment group as well as the untreated control and positive control (100 mg/kg of dimethylnitrosamine) groups. After a 16-hour incubation period the animals were sacrificed for removal of yeast cells from the peritoneum for plating and counting. Plates were incubated at 32°C for 4 to 5 days prior to mutant colony counting. Mean numbers (± S.D.) x 10^{-4} of mutant colonies were as follows: control, 1.80 ± 0.48; low dose, 1.18 ± 1.18; mid-dose, 1.03 ± 0.91; high-dose, 1.84 ± 0.28; positive control (dimethylnitrosamine 100 mg/kg 31.1 ± 3.31. As was the case for the studies above the negative result obtained by Mondino (1979) may result from TCI not being mutagenic, or it may be that the compound or its metabolites did not reach the test organisms, in the peritoneum of the test mice.

The mutagenic properties of technical grade TCI in the XV 185-14C haploid strain of *Saccharomyces cerevisiae* were assessed *in vitro* by Shahin and Von Borstel (1977). The ability of TCI to induce mutations at the *lys 1-1*, *his 1-7*, and *hom 3-10* loci was assessed in this study. Stock cultures were incubated in liquid YPG medium at 30°C until a stationary growth phase was achieved. Cells were harvested, washed, and adjusted to a population of 1×10^8 cells/ml. In order to estimate its mutagenic effect in the absence of metabolic activation, cells treated with 1.11, 5.56, 111.1, or 222.2 mM of TCI during incubation at 30°C were withdrawn at 2, 6, 24, and 48 hours for plating and counting of colonies. No precautions were reported to have been taken to prevent excessive evaporation of the compound and ensure exposure to the test organism, but the reported toxicity indicates this was not a problem and that the cells were adequately exposed. Treated cells were compared with cells from similarly maintained untreated control and positive control experiments (222.2 mM of 2-acetylaminofluorene for tests without metabolic activation and 8 ug/ml of ethyl methanesulfonate for tests with and without metabolic activation). Plates were maintained at 30°C for 5 days to estimate survival, and the assays were performed in triplicate.

In the study described above, concentrations of 111.1 mM and 222.2 mM TCI in the presence of metabolic activation increased the reversion frequencies at the *lys 1-1*, *hom 1-10*, and *his 1-7* loci (Table 7-3) but only at levels that were also highly toxic (> 99% cell killing). The revertants/10^7 survivors for *lys 1-1*, *his 1-7*, and *hom 3-10*, corresponding to 1 hour treatments of 111 and 222 mM, were 2552 and > 23,200, 3553 and > 29,000, and 5313 and > 25,000, respectively compared to negative control values of 15.3, 19.5, and 8.6. The significance of the reported increased incidences of mutations in tests conducted with

TABLE 7-3. MUTAGENICITY TECHINICAL GRADE OF TRICHLOROETHYLENE AT THE HIS 1-7 LOCUS OF *SACCHAROMYCES CEREVISIAE*
(adapted from Shahin and von Borstel 1977)

Dose (mM)	-S9 (6-hr incubation)		+S9 (4-hr incubation)	
	Revertants	%Survival	Revertants	%Survival
0	14.3	100	19.2	99
1.11	16.7	90	15.9	72
5.56	22	72	16.8	65
111.1	0	<0.13	22,000	<0.1
222.2	0	<0.013	>29,000	<0.1
Positive Control	83*	61	6300†	0.23

*2-acetylaminofluorene at 20 ug/ml.

†EMS at 8 ul/ml.

metabolic activation is uncertain because the increases were noted only when there was greater than 99% killing. The positive control in the tests with metabolic activation (EMS) was inappropriate because it is a direct-acting mutagen. TCI was ineffective as a mutagen without metabolic activation at all doses tested, but increased numbers of revertants were noted with activation. With respect to the 48-hour treatment without activation, the maximum number of revertants per 10^7 survivors for the three loci individually was 10 to 15 for untreated controls (98% survival), 12.8 to 21.4 for 1.11 mM (73% survival), 11.4 to 17.1 for 5.56 mM (64% survival), and 0 for 11.1 mM and 222.2 mM (< 0.1% survival) of TCI. The lack of revertants with the two highest concentrations appears to correspond to failure of the cells to survive after plating. The largest number of revertants per 10^7 survivors produced by 2-acetylaminofluorene (222.2 mM) was 102 (*lys 1-1*) after a 6-hour treatment, and 673 (*his 1-7*) and 488 (*hom 3-10*) after a 24-hour treatment. It may be that there is a "window" of effective concentrations where TCI is mutagenic but not excessively toxic. Such a concentration effect cannot be ruled out because levels between 5.56 mM (ineffective) and 111.1 mM (suggestive response but highly toxic) were not tested and it was in this dose range that the weak positive responses discussed below were obtained by Bronzetti et al. (1978) and Callen et al. (1980) in strain D7. It is important to note that the composition of the TCI sample used in the study by Shahin and Von Borstel (1977) was not determined, and the possible contribution of contaminants to the response cannot be ascertained or ruled out (personal communication with R.C. Von Borstel, University of Alberta). These results, do not allow a conclusive evaluation to be made.

An increase in point mutations and gene conversion by a certified ACS reagent grade of TCI in *Saccharomyces cerevisiae* strains D4 and D7 was observed by Bronzetti et al. (1978) in cell suspension and in host-mediated assays.

The authors did not report a detailed chemical analysis of the sample; however, a description of the product obtained by personal communication with Fisher Scientific Company, the producer of the test sample indicates a purity of > 99.5% and stabilization with either 25 ppm diisopropylamine or 20 ppm triethylamine. In the cell suspension experiment, strain D7 cells, which can detect mitotic gene conversion at the trp locus, point (reverse) mutation at the ilv locus, and mitotic recombination at the ade locus, were incubated in the presence of TCI with or without S9 mix derived from livers of uninduced male CD-1 mice for 4 hours at 37°C before plating on selective media (for counting of ilv revertants, ade recombinants, and trp convertants) and on complete medium (for survivor counts). The results for ilv revertants are presented in Table 7-4. The other results will be discussed later in the section on tests indicating DNA damage. In the host-mediated assay yeast cell culture was instilled into the retroorbital sinus of male adult CD-1 mice. Two experiments were included in this assay: an acute experiment in which a single gavage dose of 400 mg/kg was given immediately after introduction of strain D4 or D7 cells on the day of the assay. Mice were sacrificed 4 hours following introduction of the yeast cells and liver, lungs, and kidneys were removed for homogenization and subsequent plating of yeast cells as described for the suspension assay. Assays in this study were done in triplicate or quadruplicate, and the data for gene revertants are presented in Table 7-4 as mean ± S.E.

The results of Bronzetti et al. (1978) were as follows. In the absence of metabolic activation, 10 mM and 40 mM concentrations of TCI effected a dose-related reduction in cell viability in the cell suspension system but did not induce a mutagenic response. Survival was reduced to approximately 87% with the 10 mM dose and to 65% with the 40 mM dose compared to 100% for the controls. TCI did produce concentration-dependent increases in point mutation

TABLE 7-4. MUTAGENICITY OF REAGENT GRADE TRICHLOROETHYLENE AT THE ILV LOCUS IN *SACCHAROMYCES CEREVISIAE*
(adapted from Bronzetti et al. 1978)

Suspension Tests Strain D7

Concentration (mM)	Revertants/10^6 x ± S.E.	% Survival
Without Metabolic Activation		
0	0.75 ± 0.12	100
10	0.85 ± 0.15	87.5 ± 1.0
40	0.87 ± 0.06	64.1 ± 2.1
With Metabolic Activation		
0	0.85 ± 0.06	100
10	1.16 ± 0.16	81.6 ± 2.9
20	1.77 ± 0.12	67.1 ± 2.8
30	2.24 ± 0.17	55.5 ± 1.7
40	3.23 ± 0.32	50.3 ± 1.4

Host-Mediated Assay in CD-1 Mice

Strain	Organ	Treatment	No. of Mice	Revertants/10^6 x ± S.E.
Acute Exposure (400 mg/kg)				
D7	Liver	Control	3	0.08 ± 0.04
		TCE	4	0.56 ± 0.52
	Lungs	Control	2	0.08 ± 0.002
		TCE	3	0.40 ± 0.19
	Kidneys	Control	2	0.21 ± 0.02
		TCE	4	0.79 ± 0.37

frequencies with metabolic activation at doses of 10, 20, 30, and 40 mM with metabolic activation. Positive responses were also observed in the host-mediated assays. Fourfold to eightfold increases were obtained in the number of revertants compared to the negative controls. The highest response was observed in yeast recovered from the kidney where $0.79 \pm 0.37 \times 10^{-6}$ revertants were found in the treated animals vs. $0.21 \pm 0.02 \times 10^{-6}$ in the negative controls.

Like Bronzetti et al. (1978), Callen et al. (1980) also employed a TCI sample obtained from the Fisher Scientific Company. They used Saccharomyces cerevisiae strains D4 and D7 to assess TCI's genetic activity and strain D5 intact yeast cells or microsomal suspensions to study its metabolism indirectly. Gene conversion at the trp 5 locus, mitotic recombination at the ade 2 locus, and gene reversion of the ilv 1 locus (Table 7-5) of strain D7 were assessed by exposures of the cells to 0, 15, and 22 mM solutions in suspension at 37°C for 1 hour followed by plating on appropriate media, allowing for expression of genetic activity and, finally, scoring. A twofold increase in reverse mutations at the ilv 1 locus over the spontaneous control values was observed at 15 mM (7.4 vs. 3.6 revertants $\times 10^{-6}$ survivors, respectively) without an exogenously supplied metabolic activation system (67% cell survival). A severely toxic effect was observed at 22 mM. A positive response without activation was observed by Callen et al. (1980) but not by Bronzetti et al. (1978). The reason for this is not known, but it may be due to the stages of growth the yeast cells were in when tested. Callen et al. (1980) found TCI to be mutagenic to cells from log phase cultures but not to cells from stationary cultures of D7. Bronzetti et al. (1978) did not report the growth phase of the cells used in their test, but if they employed stationary phase cells a negative response without activation would be expected.

Callen et al. (1980) provided additional suggestive evidence that Saccharomyces

TABLE 7-5. MUTAGENICITY OF TRICHLOROETHYLENE AT THE ILV LOCUS IN *SACCHAROMYCES CEREVISIAE* D7
(adapted from Callen et al. 1980)

	Survival		ilv 1	
Concentration (mM)	Total Colonies	%	Total Revertants	Revertants 10^6 Survivors
0	1201	100	43	3.6
15	802	67	59	7.4
22	3	0.3		

can metabolize TCI. They analyzed whole cell suspensions of strain D5 in the log phase of growth in a spectrophotometer after exposure to unspecified amounts of TCI. Peaks with maximum of 450 and 505 mM were observed. The peak at 450 nm appeared to reach a maximum after roughly five minutes and after this period an additional peak at 423 nm was observed. This spectrum is similar to that observed when TCI is added to mammalian microsomes. These results indicate that trichloroethylene enters exposed yeast cells and suggest it is metabolized by a yeast cytochrome P-450-independent monooxygenase system.

The yeast studies show that commercially available TCI is mutagenic in *Saccharomyces cerevisiae*. The data are consistent with TCI metabolites being mutagenic, because the samples tested are represented as being free from epoxide stabilizers (which are direct-acting mutagens), and metabolic activation appears to be required for the genetic activity of TCI. The doses required to elicit twofold to fourfold increases in gene reversion (i.e., 10-40 mM in suspension tests and 400 mg/kg in the host-mediated assay) show the commercial samples of TCI to be weakly active. The negative responses obtained with the *Schizosaccharomyces pombe* host-mediated assays are not considered to diminish the positive results in *Saccharomyces* because of the inherent insensitivity of the *S. pombe* test (forward mutation assay with high spontaneous control levels and no selection scheme).

7.1.2.1.2 *Angiosperms*. Testing for somatic cell mutations in the stamen hairs of *Tradescantia* clone 4430, an interspecfic hybrid (T. *subacaulis* x T. *hirsutiflora*), was done by Schairer et al. (1978) to assess the mutagenic potential of TCI. A group of unrooted fresh cuttings containing young inflorescences possessing flower buds in a range of developmental stages was exposed to 0.5 ppm TCI for 6 hours. Following treatment, the cuttings were grown in aerated Hoagland's nutrient solution and analyzed each day as they bloomed for 3 weeks after treatment. One hundred forty-eight mutations were observed out of 44,000 stamen hairs scored. The mutation frequency (± S.E./100 cells), after the negative control values (not reported) were subtracted, was reported to be positive (0.112 ± 0.036; $P < 0.01$). Based on the data given, however, this value cannot be verified. The mutation frequency (± S.E./100 cells) for 6 hour exposures to 5 ppm EMS and 75 ppm vinyl chloride were 1.012 ± 0.133 and 0.112 ± 0.046, respectively. Because the purity and source of the sample were not provided, it is not possible to ascribe with certainty the observed positive effects to the genotoxic action of TCI per se.

7.1.2.2 Animals--

7.1.2.2.1 Insects (*Drosophila*). Abrahamson and Valencia (1980) fed 1000 ppm TCI in 0.2% sucrose for 72 hours to male Canton-S (wild type) *Drosophila melanogaster*, and subsequently mated 102 of these flies with female FM6 (a marked and balanced X-chromosome) flies and screened the F_2 descendents for mutagenicity using the sex-linked recessive lethal test. Ninety-six of the treated males were fertile. Administration of the test compound was performed in a glass shell vial containing filter material saturated with the feeding solution which was renewed twice daily. However, because of the volatility of TCI the actual dose given to the flies might have been much less than expected in the feeding study. Treated and untreated control males were mated individually with three females each and then transferred without anesthetization to fresh females at 2- to 3-day intervals until a total of four broods of progeny were produced. The fertile females were left in the vials for 1 week. In a simultaneous experiment, 0.3 ul of TCI was injected into the abdomen of the male flies before mating. A technical grade sample from Allied Chemical Co. was used, and the purity and composition of the sample was not ascertained (personal communications with L. Valcovic, Food and Drug Administration, sponsor of the study; and Allied Chemical Co.).

TCI was not mutagenic in the above test. The frequency of lethals (number of lethals/number of chromosomes tested) was 0.12% (19/9248) in the feeding experiment, 0.17% (4/2344) in the injection experiment, 0.31% (31/10,009) in concurrent controls, and 0.24% (230/94,491) in the historical controls used in the testing laboratory for 2 years prior to completion of this study. The negative result obtained in this test indicates that either the test substance did not reach the target site (in the testes) or that TCI is not mutagenic.

Beliles et al. (1980) also tested TCI in Drosophila for its ability to cause sex-linked recessive lethals and, in addition, for its ability to cause X or Y chromosome loss. Three-hundred one-day old B^SY y^+ac^+, $(K^S1+2)^-/X.Y^S$, INEN2,f males were placed in each of three screen cages and subsequently exposed to 0, 100, and 500 ppm TCI vapor from North Strong (estimated to be 99.9% pure by infrared spectroscopy) for 7 hours. An unspecified number of males serving as positive controls were fed 0.025 M EMS in 1% sucrose for 8 hours. Two-hundred males per treatment group were individually mated to sequential groups of 3 $In(1)sc^8$, sc^8 1 B/In(1)dl-49 B^{M1}, y ac v B^{M1} virgin females. The brooding scheme consisted of a 2-3-3-2 day mating sequence enabling specific sperm cell stages to be tested (i.e., spermatozoa, spermatid, spermatocyte, and spermatogonia). Losses of the entire X or Y chromosome and the short or long arm of the Y chromosome were detected by scoring for specific phenotypic classes in the F_1 generation. F_1 males of the genotype y ac v B^{M1}/In(1)EN2,f were mated to virgin $Y^S/X.Y^L$, In(1)dl-49, v f B females and the occurrence of recessive lethal mutations on the In(1)EN2, f chromosome were detected by the lack of males with forked bristles in the F_2 generation. The frequencies of X or Y chromosome loss were 0.07% and 0.17% for the low and high doses, respectively, compared to 0.06% for the concurrent negative control. These values are not signficantly different; however, the 0.17% was close to a significant P value.

The positive control EMS did not induce sex chromosome loss under these test conditions; thus it is not possible to determine whether the test was conducted properly. The frequencies of losses of the long arm of the Y chromosome, 0.03% and 0.05% for the low and high doses, respectively, and losses of the short arm of the Y chromosome, 0.02% for both dose levels, were not significantly different from the 0.01% value of the concurrent negative control for losses of both arms. Like the study by Abrahamson and Valencia (1980), Beliles et al. (1980) found TCI to be ineffective in causing an increase in the incidence of sex-linked recessive lethal mutations. The frequencies were 0.03% and 0.16% for the low and high doses, respectively, compared to 0% for the concurrent negative control and 0.11% of the historical negative control. The frequency of sex-linked recessive lethal mutations for the positive control (EMS) was 21.56% showing a significant positive response. No significant variations were observed among the four germ cell (brood) stages employed in any of the assays.

7.1.2.2.2 Rodents. TCI was tested in a mouse spot test by Fahrig (1977). Virgin females of the inbred C57BL/6J Han strain (a/a, wild type) were mated with males of the Han-rotation bred T-stock (a/a=non-agouti, a/a=brown, c^{ch} p/c^{ch} p=chinchilla and pink-eyed dilution; d se/d se=dilute and short ear; s/s=piebald spotting) to yield embryos of the genotype a/a, b/+, c^{ch}p/+ +, d se/+ +, s/+ (black coat, dark eyes). Each male was mated with two females. On day 11 of gestation, a single intraperitoneal (i.p.) injection of TCI (99.5% pure) was made into 50 and 26 females at doses of 140 and 350 mg/kg, respectively. Impurities detected in this test sample were 60 to 150 ppm ethanolamine, 0.0003% chloride (Cl_2), and 0.0001% free chloride (Cl-1) (personal communication with R. Fahrig, University Tubingen, West Germany). Litters were born on day 21 of gestation, and pups were examined for color spots, the mutagenic end-point, twice weekly between 2 and 5 weeks of age.

The number of offspring (number of litters) surviving for examination was 144 (23) in the untreated control group, 145 (38) in the low-dose group, and 51 (13) in the high-dose group. The number of coat color (brown or gray) spots found were: none in concurrent control pups, 2/794 historical control pups (0.25%), two low-dose pups (1.3%), and two high-dose pups (3.92%). Known mutagens including ethyl methanesulfonate were positive (26% at 0.9 mM EMS). Results of this study indicate that the TCI sample tested or one or more of its metabolites is able to produce mutations in somatic tissue of an intact mammal.

Based on the weak positive responses obtained in *Saccharomyces*, *Tradescantia*, and (57B46J) mice, *commercial samples* of TCI are judged to be capable of causing gene mutations after metabolic activation. Because of the requirement for activation, it is not likely that epoxide stabilizers present in the commercial samples account for the effect. However, because high doses of TCI were required to elicit a positive effect it must be considered to be only weakly mutagenic. The data for purified samples of TCI do not show it to be positive. However, weak, less than twofold, increases are reported with metabolic activation at high doses.

7.2 CHROMOSOME ABERRATION STUDIES

TCI has been examined for its ability to cause changes in chromosome number (chromosomal loss tests with *Drosophila*, micronucleus test in mice, and hypodiploid cells in humans), and structure (in studies employing mice, rats, and humans). The results of the chromosome loss test were described in the previous section (Beliles et al. 1980). The rest of the studies are presented below.

7.2.1 Experimental Animals

7.2.1.1 *Bone Marrow*--Beliles et al. (1980) tested TCI for its ability to cause chromosome aberrations in rat bone marrow cells. Adult male and female rats [CRL: COBS CD (SD) BR] were exposed to 0, 100, or 500 ppm TCI by inhalation both acutely and subchronically. The positive control was TEM at 0.3 mg/kg given i.p. in 0.85% saline. Acute exposure consisted of a single 7-hour session in the exposure chamber. Subchronic exposures consisted of 5 daily exposures of 7 hours. Bone marrow cells were then collected and slides prepared, coded, and 50 cells/animal scored. There was no increase in the number of aberrations in either study. The percentage of cells with aberrations were 0.4-2.4 in the negative control group compared to 0-2.7 in the TCI treated group. The positive control TEM was clastogenic causing 7.2-8.5% aberrant cells. TCI failed to cause the induction of aberrations or aneuploidy under the conditions of this study; however, it is not considered to be an adequate test of the clastogenic potential of TCI. The positive control was not appropriate because it was given by i.p. injection while TCI was administered via inhalation. In addition, the doses used may have been too low to detect weak activity. The lowest concentration in air reported to cause death in the rat is 8000 ppm/4 hrs (American Industrial Hygiene Association Journal 30:470, 1969). It would have been appropriate to have tested higher doses of TCI and to have incorporated a volatile and preferably a chemically related substance as the positive control.

7.2.1.2 Dominant Lethal Test--The precise nature of damage causing dominant lethal effects is not known, but there is a good correlation between chromosome breakage in germ cells and dominant lethal effects (Matter and Jaeger 1975). When dominant lethal effects are observed it can be concluded that the test substance reaches the gonads and likely causes genetic damage.

Slacik-Erben et al. (1980) assessed the ability of 0, 50, 202, and 450 ppm TCI in air to cause dominant lethal mutations in NMRI-Han/BGA mice. The sample contained 99.5% trichloroethylene stabilized with 100 mg/l triethanolamine. Analysis of a liquid sample also identified 5 ppm 1,2-dichloroethylene, 9 ppm cis-1,2-dichloroethylene, 80 ppm 1,1,1-trichloroethylene, 120 ppm carbon tetrachloride, 10 ppm 1,1,2-trichloroethane, and 815 ppm tetrachloroethylene. Fifty males per dose were exposed for 24 hours in a 1000 liter volume glass chamber equipped with a ventilator. The temperature was kept between 22-24°C and the humidity between 70-80%. For rapid equilibration the calculated volume of liquid TCI was placed in the chamber, and mixtures of TCI and air were streamed through the chamber at a rate of 200 (50 and 202 ppm) and 300 (450 ppm) l/hour. After the 24-hour exposure period, the male mice were mated individually with one untreated NMRI-Han female for days. At this time, the mated females were replaced by fresh virgin females who were kept for 4 days and then replaced, (and this was repeated, etc.) until each male had been mated singly to 12 females for 4 days each (up to 48 days after treatment). By this mating regimen all stages of spermatogenesis were sampled from mature sperm to gonial cells. Each pregnant female was sacrificed by cervial dislocation, dissected, and examined for dead and living implants. The results of the testing are presented in Table 7-6. Under conditions of this test, TCI was not detected as mutagenic, but there are several important points to consider with respect to this finding. The males exhibited no visible effects during the exposure, and doses up to 450 ppm did not alter the fertilization rate. Thus, the doses selected for test may not have approached the maximum tolerated dose. Even if the maximum tolerated dose was approached, the dominant lethal test is not recognized to be a sensitive test for detecting mutagens (Russell and Matter 1980) due to the high spontaneous level of dominant lethal events.

7.2.1.3 Micronucleus Test--Duprat and Gradiski (1980) used TCI in a micronucleus test. CD-1 mice were divided into nine groups, six of which were given up to 3000 mg/kg TCI (analytical grade 99.5% purity; unspecified source) in gum arabic orally in two dosings separated by 24 hours. The animals were sacrificed 16 hours later, bone marrow smears were made, stained, and polychromatic

TABLE 7-6. MUTAGENICITY OF TRICHLOROETHYLENE IN THE DOMINANT LETHAL ASSAY USING NMRI MICE
(adapted from Slacik-Erben et al. 1980)

Dose in ppm (rate)	Female Mated	Female % w/Implants	Total Implants	Total Dead Implants	Total Live Implants	Live Implants/ Female	% Dominant Lethal Factors*
(200 1/h)							
0	498	89.6	5578	443	5135	11.5	
50	482	90.1	5360	479	4881	11.2	2.6
202	493	91	5703	531	5172	11.5	0
(300 1/h)							
0	503	93.4	6142	502	5640	12.0	
450	488	92.4	5927	533	5394	11.97	0.25

*Dominant lethal factors (F_L)

$$F_L\% = 1 - \frac{\text{(live implants per female of test group)}}{\text{(live implants per female of the control group)}} \times 100$$

erythocytes (PCEs) and mature erythrocytes (MEs) were examined for the presence of micronuclei. The incidence of micronuclei in PCEs was higher in the vehicle control group (i.e., treated with 10% gum arabic solution) than in the untreated control (1.0 ± 0.5 vs. 0.5 ± 0.4%). Also, the incidence of what were considered micronuclei in PCEs and MEs from treated animals was greater than the vehicle control levels at all doses and showed a dose-response effect. At the highest dose (3000 mg/kg) the percentage of PCEs with "micronuclei" was 15.8 ± 5.7 compared to the vehicle and untreated control values presented above. The corresponding values for "micronuclei" in MEs were 3.4 ± 1.8, 0.2 ± 0.3, and 0.3 ± 0.3, respectively ($P < 0.01$). Because of their occurrence in MC the micronuclei observed by Duprat and Gradiski (1980) are likely artifactual (See discussion by Schmid 1975).

7.2.2 Humans

Konietzko et al. (1978) studied the incidence of chromosome aberrations (structural and numerical) in peripheral lymphomycytes of 28 chemical workers engaged in the manufacture of TCI. The workers ranged in age between 23 and 67 years with an average age of 42.5. Their exposures ranged in duration from 1 to 21 years; the average length of exposure was approximately 6 years. Since many of the workers had been exposed over a period of many years, it was not known what other chemical substances they may have been exposed to. Thus, the study could only address the long-term effects associated with working in a factory that manufactured TCI. A control group of 10 healthy young men working in the Institute of Human Genetics where the study was conducted and ranging in age from 15 to 45 years (average age 28.3 years) was selected for comparison. However, this group was not a matched control group. The exposed group had a frequency of 10.96 ± 4.4% (X ± SD) hypodiploid cells which was significantly elevated compared to the control group, 6.5 ± 3.2% hypodiploid cells. Concerning chromosome breaks/100 cells the frequencies were 3.1 ± 3.6 and 0.6 ± 0.69, respectively. Because the control group was not a matched control group and since the mean frequency of chromosome breaks observed in the exposed workers was within the normal range, the increased incidence of chromosome breaks was not considered significant by the authors, although they did consider the increase in hypodiploid cells significant. Because of this it was thought appropriate to further investigate factors which may have contributed to the increased incidence of hypodiploid cells. Based on the frequency of hypodiploid cells in the controls, it was calculated that individuals possessing > 13% hypodiploid cells were abnormal. Workers were divided according to whether or not they possessed > 13% hypodiploid cells and these groups of workers were subsquently further characterized (Table 7-7). Nine individuals had greater than 13% hypodiploid cells. These individuals worked under higher daily peak concentrations of TCI (as measured on three occasions over a 2-week period for 8 hours each time) than did their fellow workers. The average daily concentrations for these two groups of workers were 75 ± 14 ppm vs. 61 ± 23 ppm, respectively. These exposure levels were used as estimates of previous years of exposure. This was thought to be a good approximation of the actual exposures received because no technical changes had been made in the plants. This report may provide suggestive evidence that the exposures resulting from the manufacture of technical grade TCI may cause chromosomal loss or nondisjunction in humans. However, the lack of an appropriate matched control group and the possibility that the incidence of hypodiploid cells was due to preparation of the chromosomes rather than exposure to TCI renders this study inconclusive.

7.3 OTHER STUDIES INDICATIVE OF MUTAGENIC ACTIVITY

Additional studies have been conducted bearing on the genotoxicity of TCI. These studies do not measure mutagenic events per se in that they do not

TABLE 7-7. NUMERICAL CHROMOSOME ABERRATIONS IN TRICHLOROETHYLENE WORKERS (adapted from Konietzko et al. 1978)

	> 13% Hypodiploid Cells (n* = 9)		< 13% Hypodipoloid Cells (n* = 18)	
Parameter	Mean Value (X)	Standard Error (S_x)	Mean Value (X)	Standard Error (S_x)
Daily average concentration (ppm)	75.0	17.4	61.4	23.1
Average maximum concentration (ppm)	205.8	96.3	115.7	57.6
Exposure (years)	5.9	4.8	5.9	5.1
Total dose (ppm x day)	0.55×10^6	0.72×10^6	0.31×10^6	0.39×10^6
Age (years)	38	9.4	40.4	9.7
Alcohol (g/day)	18.9	29.3	33.6	37.5
Nicotine (cigarettes/day)	12.8	12.7	10.1	10.2

*n = Number of individuals studied.

demonstrate the induction of heritable genetic alterations, but positive results in these test systems show that DNA has been affected. Such test systems provide supporting evidence useful for qualitatively assessing genetic risk.

7.3.1 Gene Conversion in Yeast

An increase in the frequency of mitotic gene conversion has been observed after exposure to chemical mutagens as a consequence of genetic repair (Fabre and Roman 1977). Bronzetti et al. (1978) tested TCI for its ability to induce this endpoint at the *ade* and *trp* loci in strains D4 and D7 of *Saccharomyces cerevisiae*. Strain D7 cells were incubated with TCI (ranging up to 40 mM) with or without CD-1 mouse liver S9 mix for 4 hours at 37°C in a liquid suspension assay. A dose-related increase in trp^+ convertants was observed in the tests conducted with metabolic activation ($41.0 \pm 2.6 \times 10^{-6}$ convertants at 40 mM compared to $16.3 \pm 0.42 \times 10^{-6}$ convertants for the negative control). No increase was noted in the test conducted without activation. Toxicity was observed in both tests with a 40-50% reduction in cell survival at the highest dose (40 mM). Twofold to fivefold increases in the incidences of gene conversion were noted in these tests and also in the host-mediated assays where strains D4 and D7 were instilled into the retroorbital sinus of adult male CD-1 mice. TCI was given by gavage in an acute exposure experiment (single dose of 400 mg/kg) or a subacute exposure experiment (22 pretreatment dosages of 150 mg/kg 5 days/week followed by a single 400 mg/kg dose). The largest increases (fourfold over spontaneous values) were found in yeast isolated from the liver and kidney (target organs for TCI toxicity), and gene conversion frequencies were higher with subacute dosing compared to acute dosing.

Callen et al. (1980) treated D7 cells to 0, 15, and 22 mM solutions of TCI in liquid suspension tests at 37°C for 1 hour followed by plating on selective media. Cell survival was reduced by 30% at 15 mM and by > 99% at 22 mM TCI. A fivefold increase in trp convertants was detected at 15 mM compared to the negative controls (76×10^{-6} vs. 14×10^{-6}, respectively). There was also a fourfold increase in mitotic recombination at the ade 2 locus at 15 mM TCI compared to the negative controls (12×10^{-3} vs. 3×10^{-3}, respectively). These endpoints were not reported for the cultures exposed to 22 mM TCI because of the extreme toxicity at this dose. The positive responses for gene conversion and mitotic recombination in *Saccharomyces* strain D7 indicate TCI is capable of interacting with DNA in yeast.

7.3.2 Sister Chromatid Exchange (SCE) Formation

White et al. (1979) evaluated the ability of anesthetic grade TCI (source and purity not reported) to cause sister chromatid exchanges (SCE) in Chinese hamster ovary cells. The material was administered as a gas to exponentially growing cells at 0.17% of the atmosphere in the presence of 19% oxygen, 5% carbon dioxide and 75.83% nitrogen for 1 hour with S9 mix prepared from Aroclor 1254-treated male rats. After exposure, the concentration of TCI gas in the flask was measured by gas chromotography and found to be 59% of that delivered. The cells were allowed to go two rounds of cell division in the presence of bromodeoxyuridine (BrdU). Subsequently, chromosome preparations were made and stained with Giemsa. One-hundred control and 100 treated metaphases were scored for SCEs by a single observer. The number of SCEs/chromosome were 0.536 ± 0.018 and 0.514 ± 0.018, respectively. Under the conditions of this test, anesthetic grade TCI did not yield an increased incidence of SCEs. However, it should be noted that the conditions of the test may not have provided an adequate assessment of TCI's ability to cause sister chromatid exchanges. In the first place, only one dose was administered and it may have been too low. There was no indication of toxicity in the treated cells further suggesting that they did not receive an adequate dose. In the second place, no concurrent positive controls were employed to ensure that the test system was working properly. These deficiencies reduce the weight of the negative response reported by White et al. (1979).

Gu et al. (1981) collected blood from six individuals occupationally exposed to TCI, cultured their peripheral lymphocytes, and scored the chromosomes for SCEs. Blood was also collected from nine additional persons who served as the control group and as blood donors for testing trichloroethanol and chloral hydrate, metabolites of TCI, *in vitro* for the induction of SCEs. A significantly higher number of SCEs/cell was reported for the exposed group compared to the controls (9.1 ± 0.4 vs. 7.9 ± 0.2). There was no information about the levels of TCI the subjects were exposed to in the workplace, but there was a good correlation between the average number of SCEs/cell per person and the levels of trichloroacetic acid and trichloroethanol measured in the blood. Those with the highest mean value for SCEs also had the highest level of these metabolites of TCI in their blood. Cultured lymphocytes were exposed to trichloroethanol and chloral hydrate at 178 and 54.1 mg/l, respectively for 68-72 hours, and increases in SCEs were observed compared to the untreated cultures. The results are tabulated as follows:

Group	No. of Karyotypes	X ± SD	Between	from t Test *
1. Control	212	7.9 ± 3.0		
2. Trichloroethanol (1)	24	9.8 ± 3.2	1&2	<0.05
3. Trichloroethanol (2)	72	9.2 ± 3.3	1&3	<0.01
4. Chloral Hydrate	52	10.7 ± 4.0	1&4	<0.001
5. TCI *in vivo*	194	9.1 ± 4.9	1&5	<0.01

*Calculated by the authors.

The results obtained by Gu et al. (1981) are suggestive of a positive response.

The chemical substances to which the TCI workers in this study (Group 5) were exposed and the extent to which such exposures occurred are unknown. It is likely that they were exposed to other substances which have been shown to be mutagenic (e.g., 1,2-epoxybutane and epichlorohydrin) and these may be responsible for the weak effects. However, the positive responses obtained by Gu et al. (1981) in *in vitro* studies with trichloroethanol (> 99.5% pure, Fluka Groups 2 & 3) and crystalline chloral hydrate (Group 4) show that these metabolites of TCI could be responsible for the induction of SCEs in TCI workers.

7.3.3 Unscheduled DNA Synthesis (UDS)

Four tests have been performed to assess the ability of TCI to cause unscheduled DNA synthesis (Beliles et al. 1980, Perocco and Prodi 1981, Williams and Shimada 1983, and Williams 1983). Although demonstration that a chemical causes unscheduled DNA synthesis does not provide a measurement of its mutagenicity it does indicate that the material interacts with DNA.

Beliles et al. (1980) exposed human WI-38 cells to TCI at doses ranging from 0.1 to 5.0 ul/ml to evaluate its ability to cause UDS. The positive controls N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and benz(a)pyrene (BaP) were used at concentration ranges betweem 1.25 to 10 ug/ml and 2.5 to 20 ug/ml, respectively. Both agents were positive; however, BaP produced a non-linear dose-response effect. All studies were conducted at the same time on a single batch of WI-38 cells. TCI yielded a weak increase at the two lowest doses (1.5 to 1.8-fold increase) both with and without activation (Table 7-8). The response is only suggestive of an effect, but the testing done with S9 may not have provided an optimal test of its gentoxic potential because the positive control (BaP) was only effective at one dose (2.5 ug/ml); higher doses resulted in a negative response although this may have due to cell killing.

Perocco and Prodi (1981) collected blood samples from healthy humans,

TABLE 7-8. UNSCHEDULED DNA SYNTHESIS IN WI-38 CELLS
(adapted from Beliles et al. 1980)

Test	Compound Concentration (ul/ml)	DNA (ug)	DPM	DPM/ (ug DNA)	Percent of Control	Response
Nonactivation						
Solvent Control, DMSO	0	23.76	988.7	41.6	100	
MNNG	5 ug/ml	6.27	472.9	75.4	181	+
Trichloroethylene	0.1	24.75	1561.3	63.1	152	+
	0.5	23.76	1616.1	68.0	164	+
	1.0	26.07	1221.3	46.8	113	-
	5.0	13.20	150.6	11.4	27	-
Activation						
Solvent Control, DMSO	0	24.75	834.5	33.7	100	
BaP	2.5 ug/ml				170	+
Trichloroethylene	0.1	29.70	1500.3	50.5	100	-
	0.5	28.38	1622.1	57.2	170	+
	1.0	21.78	860.6	39.5	117	-
	5.0	21.78	1006.8	46.2	137	-

separated the lymphocytes, and cultured 5 x 10^5 of them in 0.2 ml medium for 4 hours at 37°C in the presence or absence of TCI (Carlo Erban, Milan, Italy or Merck-Schuchardt, Darmstadt, FRG, 97-99% pure). The tests were conducted both in the presence and in the absence of PCB-induced rat liver S9 mix. A comparison was made between treated and untreated cells for scheduled DNA synthesis (i.e., DNA replication) and unscheduled DNA synthesis. A difference was noted between the groups with respect to scheduled DNA synthesis measured as dpm of [^{3}H] deoxythymidylic acid (TdR) after 4 hours of culture (2661 ± 57 dpm in untreated cells compared to 1261 ± 36 dpm in cells treated with 5 ul/ml TCI). Subsequently, 2.5, 5, and 10 ul/ml (0.028, 0.056, and 0.11 umole/ml) TCI was added to cells which were cultured in 10 mM hydroxyurea to suppress scheduled DNA synthesis. The amount of unscheduled DNA synthesis was estimated by measuring dpm from incorporated [^{3}H]TdR 4 hours later. At 10 ul/ml TCI 392 ± 22 and 439 ± 23 dpm were counted without and with exogenous metabolic activation, respectively. Both values were lower than corresponding negative controls of 715 ± 24 and 612 ± 26 dpm, respectively. No positive controls were run to ensure that the system was working properly, although testing of chloromethyl methyl ether (CMME) with activation resulted in a doubling of dpms over the corresponding negative control values (1320 ± 57 at 5 ul/ml CMME versus 612 ± untreated). The authors calculated an effective DNA repair value (r) for each chemical based on the control and experimental values with and without metabolic activation. TCI was evaluated by the authors as positive in the test, but they did not state their criteria for classifying a chemical as positive. It is important to note that none of the experimental values from cells treated with TCI without metabolic activation had higher dpm values than the controls. Furthermore, only one out of three experimental values was greater than the controls with metabolic activation (668 ± 34 at 5 ul/ml compared to control value of 612 ±

26). The increase was not statistically significant. Thus, the positive finding reported in this work is judged to be inconclusive.

In unpublished papers, Williams and Shimada (1983) and Williams (1983) tested samples of TCI for their ability to cause unscheduled DNA synthesis in rat and mouse primary hepatocyte cultures (HPC DNA repair assay). In the Williams and Shimada (1983) report two samples were tested using rat primary hepatocytes. One was fully stabilized, and the other, referred to as "non-stabilized," contained 99.9% TCI. No information was provided about the remaining components. Two types of tests were conducted. One was a conventional HPC DNA repair assay in that the hepatocytes were exposed to TCI added to the culture medium. The other was a modified assay, and the cells were placed in small glass dishes in a sealed incubator and exposed to TCI vapor. Tritiated thymidine (^{3}H-TdR) was added to the culture medium in both types of tests. If TCI caused DNA damage repaired by the excision repair pathway, ^{3}H-TdR would be incorporated into the repaired DNA. To detect such incorporation, the cells were fixed on glass slides and subsequently coated with radiotrack emulsion. After an incubation period the slides were developed and examined microscopically to count developed silver grains in the radiotrack emulsion over the cell nuclei.

Williams and Shimada (1983) used 2-acetylaminofluorene and vinyl chloride as positive controls for the conventional and modified exposure assays, respectively. 2-acetylaminofluorene was highly active (170 grains/nucleus at 10^{-5}M) and by comparison vinyl chloride was weakly active at 2.5% (5 grains/nucleus) and 5% (11 grains/nucleus) in increasing unscheduled DNA synthesis compared to the negative controls (3 grains/nucleus).

In the modified HPC DNA repair assay, cells were exposed to dosages up to 2.5% TCI in the air for 3 or 18 hours. No increase in the grain count was observed (the number of grains/nucleus ranged from 0.2 ± 0.2 to 3.2 ± 2.5 in treated cells compared to 2.75 ± 2.76 in the untreated controls). Similarly, negative results were reported in the conventional assay where the cells were exposed to concentrations as high as 1% TCI in the medium.

There are several factors to consider in the analysis of this assay.

First, the system is designed to detect the occurrence of a specific type of repair (i.e., "long-patch" excision repair). Xenobiotics which cause damage that is not repaired or that is repaired by another mechanism will not be detected as possessing genotoxic activity in the system.

Second, the endpoint measured may not actually reflect repair synthesis of chromosomal DNA in the nucleus (Lonati-Galligani et al. 1983). Enhanced incorporation of ^{3}H-TdR into mitochondrial DNA or suppressed ^{3}H-TdR incorporation into mitochondrial DNA without a concomitant increased or decreased incorporation into nuclear DNA can lead to false negative or positive results, respectively. Thus, both nuclear incorporation and cytoplasmic incorporation should be measured and the dose-responses plotted for each to serve as a basis for deciding whether or not the compound has induced UDS.

Third, nuclear grain counts vary with nuclear size (Lonati-Galligani et al. 1983); thus, it may be appropriate to express grain counts as a percentage of the measured nuclear area. In the studies by Williams and Shimada (1983) the values were transformed by subtracting cytoplasmic grain counts from total nuclear grains counts to give a net grain count; as a result the data are difficult to evaluate, and the way they are presented may preclude the detection of weakly active agents. Thus, although the results of this study are negative they do not provide convincing evidence that TCI does not induce UDS. This is a relevant point because vinyl chloride, a structurally related compound and known mutagen, was only weakly active in this test and then only at doses as high or higher than the highest dose tested for TCI.

In the Williams (1983) study, TCI (source and purity not reported) was assayed in the HPC DNA repair test using a conventional liquid exposure protocol. Primary hepatocytes from B6C3F1 mice and Osborne-Mendel rats were tested in two separate test series. A positive dose-related response was obtained in the mouse cells between 10^{-4}M and 10^{-2}M (18.24 ± 3.95 grains/nucleus at 10^{-2}M vs. 0.78 ± 1.18 grains/nucleus in the concurrent negative control). TCI was reported to be nontoxic between 10^{-6}M and 10^{-2}M. A negative response was obtained in testing conducted in rat cells. Positive and negative controls were employed for both sets of experiments and responded appropriately, but the concerns noted above regarding the Williams and Shimada (1983) study apply here as well. In spite of this, the positive response in the mouse cells shows that a commercially available sample of TCI is genotoxic in B6C3F1 mouse liver cells.

The positive responses for gene conversion and mitotic recombination in yeast, and UDS in mouse cells provide evidence that commercially available TCI reaches and is weakly active in damaging DNA.

7.4 EVIDENCE THAT TCI REACHES THE GONADS

Although not bearing on the ability of TCI to cause mutations, two studies have been conducted to assess its ability to induce sperm morphological abnormalities in mice (Land et al. 1979 and Beliles et al. 1980). A statistically significant increase was observed in both studies strongly suggesting that the compound or an active metabolite reaches the gonads. Land et al. (1979) exposed groups of $(C57BL/C3H)F_1$ male mice (13 weeks old) to air (negative control), 0.02% and 0.2% anesthetic grade TCI vapor 4 hours/day for 5 consecutive days. Twenty-eight days after the first day of exposure the animals were sacrificed. Both cauda epididymides were removed, minced, strained, and stained in 1% Eosin Y. Slides were prepared, mounted, coded, and evaluated by scoring 1000 sperm/slide at

400 X magnification. Data were recorded as percent abnormal sperm. The means were calculated for each group and compared with controls using the sample t-test. As noted below, TCI exposure resulted in statistically significant elevations in the percentage of morphologically abnormal sperm.

Agent	Concentration %	Number of Animals Studied	% Abnormal Sperm ± S.E.
Air		15	1.42 ± 0.08
Trichloroethylene	0.02	5	1.68 ± 0.17
	0.2	5	2.58 ± 0.29*

*Statistically significant difference compared to controls ($P < 0.001$).

This study provides suggestive evidence that exposure to a high concentration of TCI, during the period of meiotic division damages early spermatocytes.

Beliles et al. (1980) also observed a significant increase in sperm head abnormalities in male CD-1 mice but not albino rats [CRL: COBS CD(SD) BR]. Groups of 12 animals were dosed 7 hours/day for 5 days to 0, 100, or 500 ppm TCI. After dosing, groups of four animals were killed at the end of 1 week, 4 weeks, and 10 weeks. Sperm was collected from the caudae epididymides and suspended in 0.85% saline, stained, and scored for abnormalities. At least 2000 sperm were analyzed/group (500/animal). The negative responses obtained in the rat study are not considered adequate for assessing the ability of TCI to reach the testes because the positive controls gave a negative response. Increased incidences of abnormal sperm were observed in the mice of the 500 ppm group compared to the negative controls for weeks 1 and 4 (i.e., 18.9% vs. 6.8% and 23.5% vs. 8.1%, respectively). A significant increase was also noted in the 100 ppm exposure group compared to the negative control for week 4 (14.8% vs. 8.1%).

7.5 MUTAGENICITY OF METABOLITES

Trichloroethylene is metabolized to trichloroethanol, trichloroethanol glucuronide, and trichloroacetic acid. There are several intermediates leading to the formation of these end products of metabolism, including some which have been identified in man (e.g., chloral hydrate) and some of which have not been identified but which are thought to occur (e.g., trichloroethylene oxide). Some mutagenicity studies bearing on a few of TCI's metabolites are discussed below. See the chapter on metabolism for a more detailed discussion.

Kline et al. (1982) tested the mutagenic potential of TCI oxide in Salmonella strain TA1535 and E. coli strain WP2 uvrA. They observed no increases in revertant colonies at doses ranging from 0.25 to 5 mM. Preincubation tests were conducted and toxicity was observed at the highest doses demonstrating that the bacteria were exposed to sufficient concentrations of the test material. In the same paper epoxide metabolites of other chloroalkenes were found to be mutagenic under the same conditions of test (i.e., cis- and trans-1-chloropropene oxide, and cis- and trans-1,3-dichloropropene oxide). The genotoxicity of these compounds was also assessed by differential growth inhibition of the DNA polymerase-deficient E. coli mutant $polA^-$ compared to its polymerase proficient $polA^+$ parent. TCI oxide was positive in this assay yielding a dose-related decrease in the survival of $polA^-$ cells (up to 40%) compared to the $polA^+$ cells at doses ranging from 0.006 to 0.11 mM/ml.

Both Waskell (1978) and Gu et al. (1981) assessed trichloroethanol for its ability to cause genetic effects. As discussed in the section on gene mutation studies in Salmonella (using TA98 and TA100), Waskell (1978) tested trichloroethanol at doses up to 7.5 mg/plate and found no increases in the revertants compared to the negative controls.

Gu et al. (1981), on the other hand, found suggestive evidence that trichloroethanol (at 178 mg/l) may be an inducer of SCEs in primary cultures of human lymphocytes. In two experiments (of 24 and 72 karyotypes analyzed respectively), Gu et al. (1981) found 9.8 ± 3.2 and 9.2 ± 3.3 SCEs/nucleus compared to the control value of 7.9 ± 3.0.

Trichloroacetic acid and chloral hydrate were also tested by Waskell (1978) in the study mentioned above. Trichloroacetic acid at 0.45 mg/plate was not mutagenic to either TA98 and TA100. Chloral hydrate, however, was weakly active yielding consistent but always less than twofold increases in the number of TA100 revertants compared to the spontaneous level over a dose range from 0.5 to 10 mg both with and without metabolic activation. These increases suggest chloral hydrate is mutagenic to Salmonella strain TA100.

In their testing of chloral hydrate Gu et al. (1981) found that chloral hydrate at 54.1 mg/l caused increases in the number SCEs in cultured human lymphocytes compared to untreated control cells (i.e., 10.7 ± 4.0 vs. 7.9 ± 3.0). Again the response was suggestive of an effect.

The weak increases obtained with trichloroethylene oxide, trichloroethanol, and chloral hydrate are consistent with the hypothesis that they may be responsible for the mutagenic effects observed with TCI after metabolic activation.

7.6 BINDING TO DNA

Stott et al. (1982) studied the pharmacokinetics and macromolecular interactions of TCI in B6C3F1 male mice and male Osborne-Mendel rats. As part of their study, mice were given 1200 mg/kg [^{14}C] TCI (> 500 uCi/animal, specific activity = 2.4 mCi/mmol) in corn oil. Five hours later, the animals were killed, their livers were removed and frozen, and DNA was extracted two days later. After enzymatic digestion, fractions corresponding to nucleosides or purine and pyrimidine bases were separated and identified by high-pressure liquid chromatography. Aliquots were placed in scintillation fluid and the ^{14}C content was determined by liquid scintillation counting. Anaylsis of the data indicated that the ability of TCI to alkylate DNA was low. A maximum estimate of the average DNA alkylation level was 0.62 ± 0.42 alkylations/10^6 nucleotides. These data suggest TCI or its metabolites are capable of binding to DNA, but only to a limited extent, and are consistent with the suggestive mutagenic responses reported for TCI and certain of its metabolites.

7.7 SUMMARY AND CONCLUSIONS

Commercially available samples of TCI have been shown to cause responses suggestive of a positive effect in gene mutation studies using bacteria, fungi, higher plants, and somatic cells of mice *in vivo*. These responses occurred with metabolic activation only, suggesting the involvement of one or more metabolites of TCI. This requirement for metabolic activation argues against the possibility that epoxide stabilizers found in many commercial samples are responsible. The marginally increased incidences of revertant counts were only observed at high doses. Thus, commercially available TCI is only weakly mutagenic at most. TCI was not shown to cause structural chromosomal aberrations in the one test conducted to assess this endpoint.

Other tests provide evidence that commercially available TCI damages DNA. Suggestive and weak-positive responses have been observed in yeast (gene conversion and mitotic recombination), mice (UDS), and humans (SCE and UDS). Metabolic activation was required to obtain the positive responses. Certain metabolites of TCI have been tested for their mutagenic potential, and suggestive positive effects have been shown. TCI or a metabolite(s) may be capable of binding to DNA, but only minimally.

TCI causes weak increases in morphologically abnormal sperm providing evidence that its reaches the gonads. A synopsis of the results of these studies is presented in Table 7-9.

The available data provide suggestive evidence that commercial-grade TCI is a weakly active indirect mutagen, causing effects in a number of different

TABLE 7-9. SUMMARY OF TESTS FOR MUTAGENICITY OF TCI

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
I. Gene Mutations	*Salmonella typhimurium*	Reverse mutations *in vitro*	Technical-grade	-	No control to test effectiveness of S9 mix. No precautions to prevent evaporation.	Henschler et al., 1977
		Plate incorporation tests	Technical-grade	+	Two-fold increase	Margard, 1978
			Purified	-		Margard, 1978
			Anesthetic-grade	-		Waskell, 1978
			Purified^{a}	*	1.8-fold increase	Bartsch et al., 1979
		Vapor exposure	Purified^{a}	*	1.3-fold increase	Baden et al. 1979
			Reagent^{a} grade	*	1.7-fold increase	Simmon et al., 1977
	Escherichia coli	Forward and reverse mutations	Analytical-grade	*	Positive for reverse mutations only at arg locus (two-fold increase).	Greim et al. 1975
	Schizosaccharomyces pombe	Forward mutations (Host-mediated assays)	Technical-grade	*	1.7-fold increase	Loprieno et al., 1979

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

TABLE 7-9. (continued)

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
	Saccharomyces cerevisiae	Reverse mutations (*in vitro*)	Purified	-		Loprieno et al., 1979
			Purified	-	Epichlorohydrin and epoxybutane were also negative.	Rossi et al., 1983
			Purified[a]	-		Mondino, 1979
			Technical-grade	x	High toxicity	Shahin and Von Borstel, 1977
			ACS regeant-grade	+	Four-fold increase both host-mediated assay and liquid suspension test.	Bronzetti et al., 1978
			Technical-grade	+	Two-fold increase	Callen et al., 1980
	Tradescantia	Forward mutations	Unknown	*		Schairer et al., 1978
	Drosophila melanogaster	Sex-linked recessive lethals	Technical-grade	-		Abrahamson and Valencia, 1980
			Technical-grade	-		Beliles et al., 1980
	Mouse	Spot test	Technical-grade	+	Six-fold increase	Fahrig et al., 1977

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

TABLE 7-9. (continued)

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
II. Chromosomal Aberrations	*Drosophila melanogaster*	Chromosome loss	Technical-grade	-		Beliles et al., 1980
	Rat	Bone marrow	Technical-grade	x	Positive control given by different route of exposure. Doses of TCI may have been too low.	Beliles et al., 1980
	Mouse	Dominant lethal	Purified^a	-		Slacik-Erben et al., 1980
		Micronucleus	Analytical-grade	x	Positive response reported by authors may be due to artifacts in mature erythrocytes.	Duprat and Gradiski, 1980
	Human	Breaks	Occupational exposure	-		Konietzko et al., 1978
		Hypodiploid cells	Occupational exposure	x	Unmatched control group. Hypodiploid cells can be caused by preparation of chromosomes.	Konietzko et al., 1978
III. Other Studies Indicative of Mutagenic Damage	*Saccharomyces cerevisiae*	Gene conversion	ACS reagent-grade	+	Two-fold increase with metabolic activation.	Bronzetti et al., 1978

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

TABLE 7-9. (continued)

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
	Mouse	Mitotic recombination	Technical^a-grade	+	Five-fold increase	Callen et al., 1980
			Technical^a-grade	+	Four-fold increase	Callen et al., 1980
		Sister chromatid exchange	Anesthetic-grade	-	No positive controls. No evidence of toxicity.	White et al., 1979
	Human	Sister chromatid exchange	Occupational exposure	*	Increases correlated with presence of TCI metabolites trichloroethanol and trichloroacetic acid in the blood.	Gu et al., 1981
		Unscheduled DNA synthesis	Technical-grade	*	1.5 to 1.8-fold increases	Beliles et al., 1980
			Technical-grade	x		Perocco and Prodi, 1981
	Rat	HPC DNA repair assay	Stabilized	-	Vinyl chloride only weakly active.	Williams and Shimada, 1983
			Unstabilized	-		Williams and Shimada, 1983
			Technical-grade	-		Williams, 1983

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

TABLE 7-9. (continued)

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
	Mouse	HPC DNA repair assay	Technical-grade	+	8- to 20-fold increases	Williams, 1983
IV. Evidence TCI Reaches the Gonads	Mouse	Morphological sperm abnormalities	Anesthetic-grade	*	1.8-fold increase	Land et al., 1979
			Technical-grade	+	Three-fold increase	Beliles et al., 1980
V. Mutagenicity of Metabolites						
A. TCI-oxide	Salmonella typhimurium	Reverse mutations		-		Kline et al., 1982
	Escherichia coli	Reverse mutations		-		Kline et al., 1982
		Differential killing of repair deficient bacteria		+	40 % decreases in survival of Pol- vs. Pol+ cells	Kline et al., 1982
B. Trichloroethanol						
	Salmonella typhimurium	Reverse mutations		-		Waskell, 1978
	Human lymphocytes	Sister chromatid exchange		*		Gu et al., 1981

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

TABLE 7-9. (continued)

Test Category	Organism	Type of Test	Purity of TCI	Results	Comments	Reference
C. Trichloroacetic Acid						
	Salmonella typhimurium	Reverse mutations		-		Waskell, 1978
D. Chloral Hydrate						
	Salmonella typhimurium	Reverse mutations		*	1.6-fold increase	Waskell, 1978
	Human lymphocytes	Sister chromatid exchange		*		Gu et al., 1981

+ = Positive
- = Negative
* = Suggestive
a = No detectable epoxides
x = Inconclusive

test systems representing a wide evolutionary range of organisms, including humans. Based on this, *commercial* TCI may have the potential to cause weak or borderline increases above the spontaneous level of mutagenic effects in exposed human tissue. The observations that TCI causes adverse effects in the testes of mice suggest TCI may cause adverse testicular effects in man, too, provided that the pharmacokinetics of TCI in humans also results in its distribution to the gonads. The data on pure TCI do not allow a conclusion to be drawn about its mutagenic potential. However, mutagenic potential cannot be ruled out. If it is mutagenic, the available data suggest TCI would be a very weak, indirect mutagen.

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8. CARCINOGENICITY

8.1 ANIMAL STUDIES AND CELL TRANSFORMATION STUDIES

There have been a number of laboratory investigations of the carcinogenic potential of trichloroethylene (TCI) in experimental animals. These studies have been done using rats, mice, and hamsters, with TCI administered by inhalation, gavage, subcutaneous injection, and topical application. These results are summarized in Table 8-1. Of the studies done, the evidence for the carcinogenicity of TCI consists of statistically significant increases of hepatocellular carcinomas in male and female B6C3F1 mice [National Toxicology Program (NTP) 1982, National Cancer Institute (NCI) 1976, Bell et al. 1978], malignant lymphomas in female NMRI mice (Henschler et al. 1980), and renal adenocarcinomas, by life table and incidental tumor tests, in male Fischer 344 rats (NTP 1982). However, inadequacies in the NTP study in rats and the IBT study in mice and limitations in the interpretation of the data in the Henschler et al. study in mice are evident, as discussed herein.

8.1.1 Oral Administration (Gavage): Rat

8.1.1.1 National Toxicology Program (NTP 1982)--A carcinogenicity bioassay on TCI in Fischer 344 rats was recently completed for the National Toxicology Program, and a 1982 draft report of the study has been reviewed by its Board of Scientific Counselors.

The TCI test sample was described as a high-purity "Hi-Tri" product obtained in two lots, one being Dow Chemical Company Lot No. TB05-206AA, used for a preliminary dose-finding study and for the first 7 months of the bioassay, and the other being Missouri Solvents Lot No. TB08-039AA, used for the duration of the bioassay. Samples of TCI were chemically analyzed by gas-liquid chromatography,

TABLE 8-1. TCI CARCINOGENICITY BIOASSAYS IN ANIMALS

Study	TCI chemical purity	Species	Dose levels, route	Results
NTP 1982	Purified	Mice, B6C3F1 Males Females	1000 mg/kg/day gavage, 103 wk	Treatment-related hepatocellular carcinomas in males and females
		Rats, Fischer 344 Males Females	500, 1000 mg/kg/day gavage, 103 wk	Renal adenocarcinomas in treated males
NCI 1976	Technical grade	Mice, B6C3F1 Males Females	1119, 2339 mg/kg/day 869, 1739 mg/kg/day gavage, 78 wk	Treatment-related hepatocellular carcinomas in males and females
		Rats, Osborne-Mendel Males Females	549, 1097 mg/kg gavage, 88 wk	Negative
Bell et al. (MCA) 1978	Technical grade	Mice, B6C3F1 Males Females	100, 300, 600 ppm inhalation, 24 mo	Increased incidence of hepatocellular carcinomas in males and females with dose
		Rats, Charles River Males Females	100, 300, 600 ppm inhalation, 24 mo	Negative
Maltoni 1979	Purified	Rats, Sprague-Dawley Males Females	250, 50 mg/kg gavage, 52 wk	Negative
Henschler et al. 1980	Purified	Mice, Han:NMRI Males Females	100, 500 ppm inhalation, 78 wk	Increased incidence of malignant lymphomas in females
		Rats, Han:Wist Males Females	100, 500 ppm inhalation, 78 wk	Negative
		Hamsters, Syrian Males Females	100, 500 ppm inhalation, 78 wk	Negative

(continued on the following page)

TABLE 8-1. (continued)

Study	TCI chemical purity	Species	Dose levels, route	Results
Van Duuren et al. 1979, 1983	Purified	Swiss mice ICR/Ha Female	1 mg, 3x/wk, 581 d topical	Negative
		Female	1 mg, 3x/wk, 14 d 2.5 ug phorbol myristate acetate, topical 452 d	Negative
		Female	0.5 mg sc/wk, 622d	Negative
		Female Male	0.5 mg, once wk gavage, 622 d	Negative
	Purified TCI epoxide	Female	1 mg TCI epoxide, 3x/wk, 2.5 ug phorbol myristate acetate, topical, 452 d	Negative
		Female	2.5 mg TCI epoxide 3x/wk, topical for 526 d	Negative
		Female	0.5 mg TCI epoxide, once wk, sc, 547 d	Negative
NTP 1982	Purified	Rats, Osborne-Mendel Marshall 540, August 28807, ACI	Gavage, 104 wk	In progress
Maltoni 1979	Purified	Mice, B6C3F1 Swiss albino	Inhalation, 78 wk	In progress
		Rats, Sprague-Dawley	Inhalation, 104 wk	
Henschler et al. 1980	Purified and stabilized	Swiss mice ICR/Ha	Gavage, 78 wk	In progress

mass spectroscopy, infrared spectrophotometry, and nuclear magnetic resonance spectroscopy. Analytical results showed a TCI purity of greater than 99.9%. Epichlorohydrin was not found by gas chromatography-mass spectroscopy at a detection level of 0.001% (v/v). The TCI product contained 8 ppm of diisopropylamine as a stabilizer. Refrigeration at 4°C created no analytically detectable decrease in purity over the course of product usage.

Stock solutions of TCI in corn oil were made once weekly for the first 16 weeks and once monthly for the remainder of the bioassay. Analysis by gas chromatography showed that these solutions were stable for 7 days at room temperature and for 4 weeks at 4°C. Stock solution levels of TCI were within 10% of nominal concentrations.

Based on survival, body weights, and pathology in a preliminary 13-week dose-finding study, estimated maximally tolerated and one-half maximally tolerated doses were selected for the bioassay. For the bioassay, 50 males and 50 females per group were given nothing (untreated controls), corn oil alone (vehicle-controls), 500 mg/kg/day of TCI in corn oil (low dose), or 1,000 mg/kg/day of TCI in corn oil (high dose). Rats were dosed daily, 5 days per week, for 103 weeks. The volume of administered corn oil was 1 ml.

Rats were randomly assigned to dose groups when 8 weeks old at the start of the study. The animals were caged in groups of 5, and decedents were replaced during the first 1.5 weeks. Temperature and humidity in the animal quarters were 22 to 24°C and 40 to 60%, respectively, and there were 10 to 15 room air exchanges every hour.

The animals were observed daily. Body weights were recorded weekly for the first 12 weeks and monthly thereafter. Each animal was necropsied, and tissues and organs, as well as all lesions and tumors, were evaluated histopathologically.

Terminal sacrifice of survivors occurred at 111 to 115 weeks.

Body weight trends in Figure 8-1 show reduced mean weight gains in high-dose males and in both treatment groups of females. A dose-related effect on mean body weights in females became evident after 60 weeks, and mean weight gain between vehicle-control and low-dose males was similar (mean body weights at the start of the study were 161 and 141 g for vehicle-control and low-dose males, respectively).

A dose-related reduction in survival of treated male rats and a smaller decrease in survival of high-dose females were evident through survival probabilities estimated by the Kaplan and Meier (1958) technique (Figure 8-2). Differences in survival among female groups were not significant ($P < 0.05$); however, survival was significantly ($P < 0.05$) lower in low-dose males ($P = 0.005$) and high-dose males ($P = 0.001$) compared to vehicle-control males. The following numbers of rats which were killed by "gavage error" were censored from the survival curves in Figure 8-1: 1 vehicle-control male, 3 low-dose males, 10 high-dose males, 2 vehicle-control females, 5 low-dose females, and 5 high-dose females. The numbers of rats alive at terminal sacrifice were as follows: 35/50 vehicle-control males, 20/50 low-dose males, 16/50 high-dose males, 36/50 vehicle-control females, 33/50 low-dose females, and 26/50 high-dose females.

It is indicated in the NTP (1982) report that, because of reduced survival in treated male rats, the statistical methods used which adjust for intercurrent mortality (life table analysis, incidental tumor test) would provide more meaningful results, statistically, than unadjusted statistical methods (Fisher Exact Test, Cochran-Armitage Trend Test). Kidney tumor data in Table 8-2 show a significant ($P < 0.05$) increase in kidney tubular adenocarcinoma incidence in high-dose males (3/16) compared to vehicle-control males (0/33) at terminal

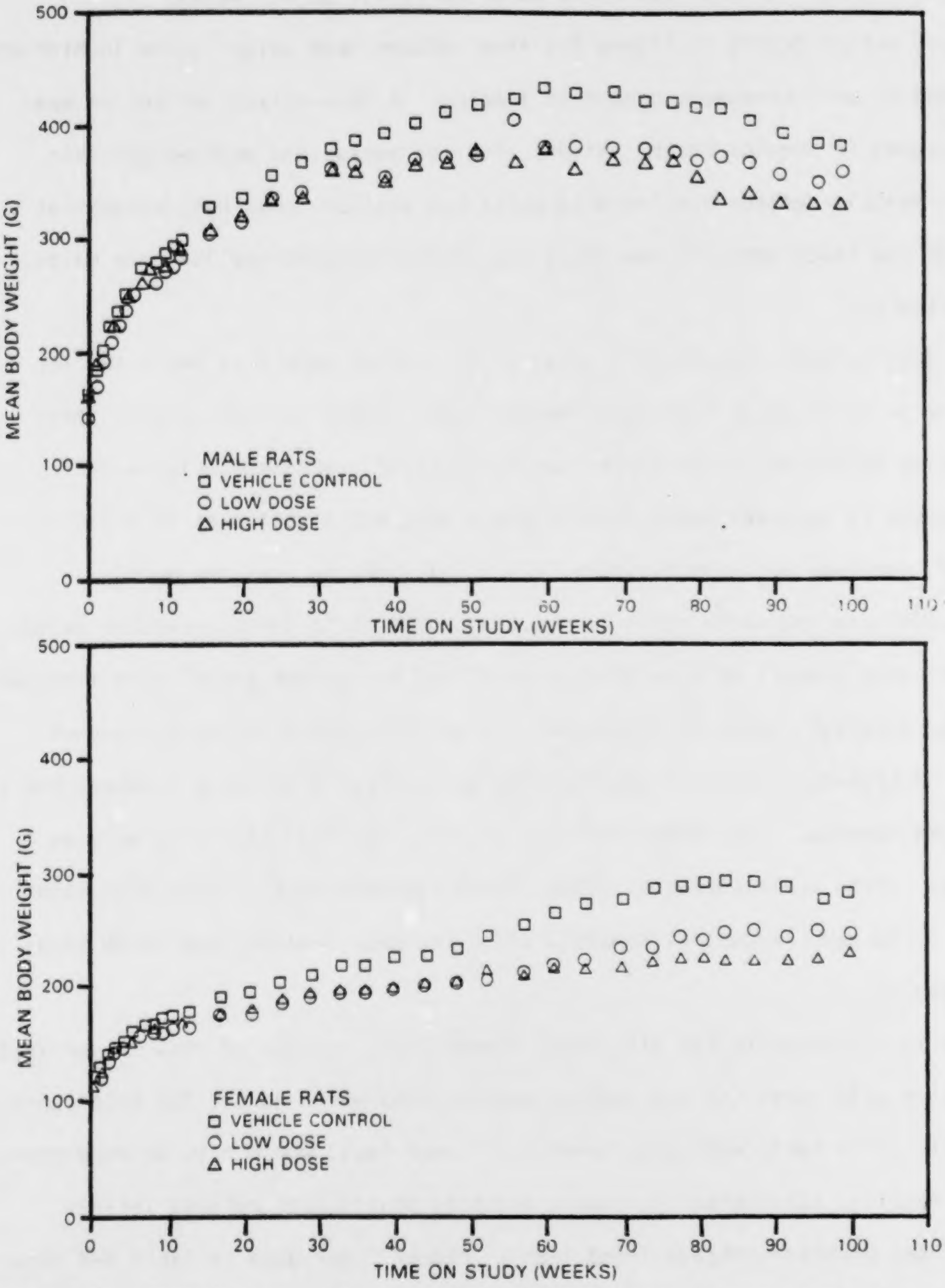

Figure 8-1. Growth curves for rats administered trichloroethylene in corn oil by gavage. (NTP 1982)

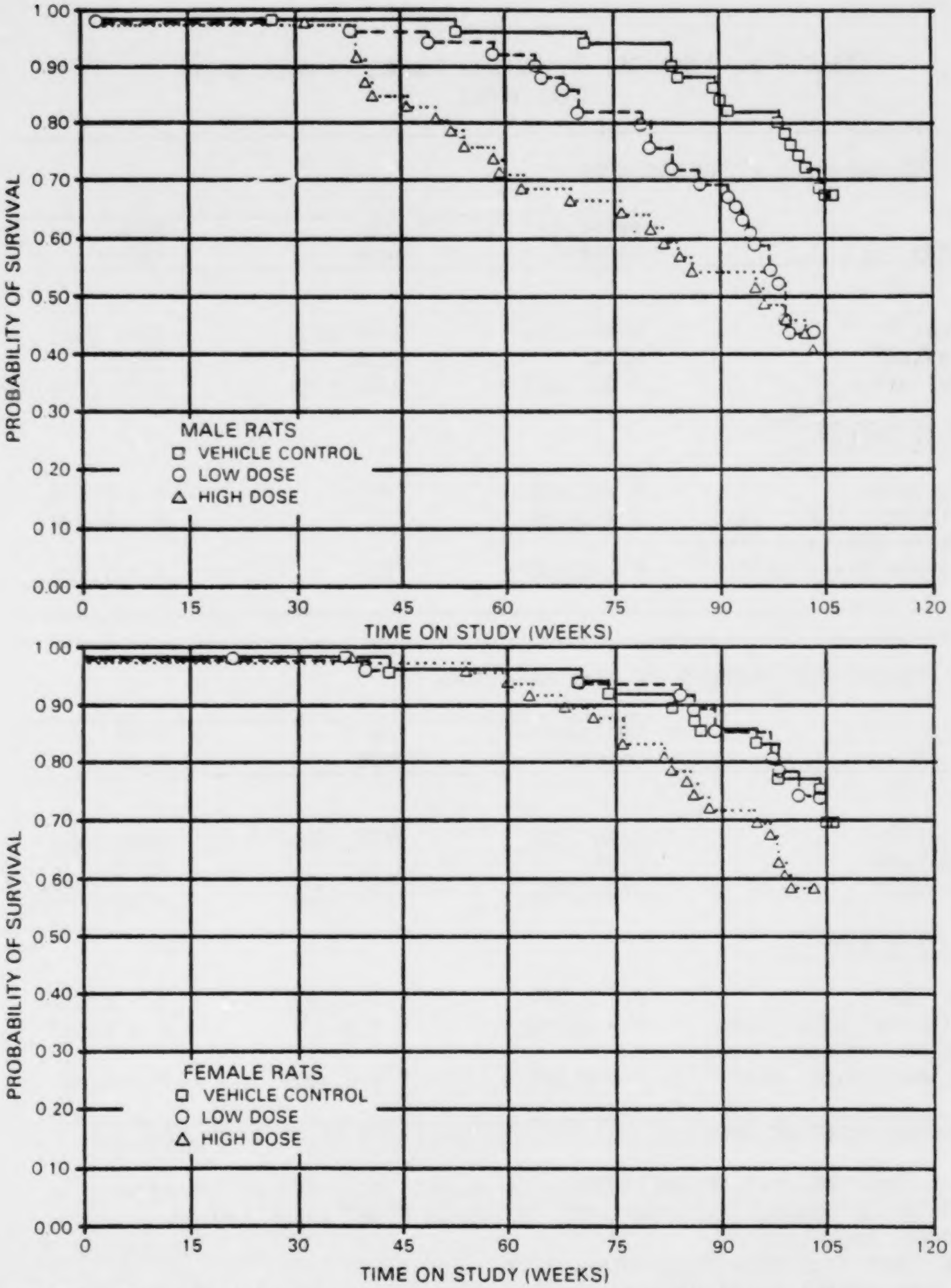

Figure 8-2. Survival curves for rats administered trichloroethylene in corn oil by gavage. (NTP 1982)

TABLE 8-2. ANALYSIS OF PRIMARY TUMORS IN MALE RATS[a]
(NTP 1982)

KIDNEY: TUBULAR-CELL ADENOCARCINOMA

Tumor rates	Vehicle-control	Low dose	High dose
Overall[b]	0/48 (0%)	0/49 (0%)	3/49 (6%)
Adjusted[c]	0.0%	0.0%	18.8%
Terminal[d]	0/33 (0%)	0/20 (0%)	3/16 (19%)
Statistical tests[e]			
Life table	P = 0.009	(f)	P = 0.028
Incidental tumor test	P = 0.009	(f)	P = 0.028
Cochran-Armitage Trend, Fisher Exact Tests	P = 0.038	(f)	P = 0.125

KIDNEY: TUBULAR-CELL ADENOMA OR ADENOCARCINOMA

Tumor rates	Vehicle-control	Low dose	High dose
Overall[b]	0/48 (0%)	2/49 (4%)	3/49 (6%)
Adjusted[c]	0.0%	5.6%	18.8%
Terminal[d]	0/33 (0%)	0/20 (0%)	3/16 (19%)
Statistical tests[e]			
Life table	P = 0.019	P = 0.194	P = 0.028
Incidental tumor test	P = 0.030	P = 0.327	P = 0.028
Cochran-Armitage Trend, Fisher Exact Tests	P = 0.084	P = 0.253	P = 0.125

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[a] Dosed groups received doses of 500 or 1,000 mg/kg of trichlorethylene by gavage.

[b] Number of tumor-bearing animals/number of animals examined at the site.

[c] Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.

[d] Observed tumor incidence at terminal kill.

[e] Beneath the control incidence are the P-values associated with the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Cochran-Armitage Test and the Fisher Exact Test compare the overall incidence rates directly.

[f] The configuration of tumor incidences precludes the use of this statistic.

sacrifice by life table ($P = 0.028$) and incidental tumor ($P = 0.028$) tests. Each test for linear trend was significant ($P < 0.05$), as shown in Table 8-2. Historical corn oil control data for male Fischer 344 rats evaluated in the NTP bioassay program as of June 15, 1981 for studies of at least 104 weeks showed a renal tumor incidence of 3/748 (0.4%) adenocarcinomas; thus, this tumor type is rare in this strain of rats. However, the comparison for kidney adenocarcinomas between high-dose and vehicle-control male rats was not significant ($P < 0.05$) by the Fisher Exact Test, and the statistical significance achieved with the other tests above reflects the significantly ($P \leq 0.005$) higher mortality in high-dose males as compared to vehicle-control males. Other observed kidney tumors in rats include a transitional cell carcinoma of the renal pelvis in a low-dose male, tubular adenomas in two low-dose males, a carcinoma (NOS) of the renal pelvis in a high-dose male, a transitional cell papilloma of the renal pelvis in an untreated control male, and a tubular adenocarcinoma in a high-dose female.

Renal tubular adenocarcinomas had a general histopathologic appearance of solid sheets of cells, often atypical, and small areas of necrosis. These tumors commonly invaded surrounding normal tissue. Small renal tubular adenomas consisted of solid collections of tubular epithelial cells filling contiguous tubules. In larger adenomas there was greater evidence of cellular atypia, and there was no sharp demarcation between larger adenomas and adenocarcinomas. Renal adenocarcinomas were distinguished from renal adenomas by basement membrane invasion.

Toxic nephrosis characterized as cytomegaly was found in 98 treated male rats and 100 treated female rats, but not in control rats. This lesion was found in early deaths, and it progressed in severity during the course of the study. Enlargement of tubular cells was detected early and progressed to the extent that some tubules were dilated and hard to identify. With longer survival the number of dilated cells decreased, corresponding tubules became dilated, and the basement membrane had a striped appearance. The most advanced stage of cytomegaly extended into the renal cortex. Cytomegaly was graded as: 1 - slight, a subtle change in a limited area of the kidney; 4 - severe, an obvious lesion which involved a substantial part of the kidney, and which could alter its function; 2 - moderate; 3 - well marked. Average grades for each group of rats were: 0 for male and female controls, 2.8 for low-dose males, 3.1 for high-dose males, 1.9 for low-dose females, and 2.7 for high-dose females. In contrast, renal nephropathy, a common spontaneous lesion in aging rats, was of lower incidence in treated males (88% in untreated controls, 85% in vehicle controls, 59% in low-dose, and 37% in high-dose) which could relate to the higher mortality in treated males.

Increases of tumor incidences at other sites in treated rats were not apparent. Malignant mesothelioma incidence was higher in low-dose males [vehicle-controls, 1/50; low-dose, 5/50 ($P = 0.042$, life table test); high-dose, 0/48], but high-dose animals did not develop this tumor type, and the low-dose incidence was not significantly ($P < 0.05$) increased according to the other statistical tests used in this study.

The TCI doses used in this study produced statistically significant decreases in the survival of male rats, and a decreased survival in high-dose female rats compared to matched vehicle-controls was apparent, although this difference was not found to be statistically significant. By definition (NCI 1976) the maximum tolerated dose in carcinogenicity bioassays is the highest dose that can be administered during the chronic study which will not be expected to alter the animals' survival rate from effects other than carcinogenicity. Thus, TCI doses beyond that maximally tolerated, particularly in male rats, appear to have been used in this study. The toxicity of TCI in the kidney tubule region, as cytomegaly, approximated the "well-marked" grade on the average in both groups of treated male rats and the high-dose group of female rats, and it has been noted that the severity of this lesion increased with longer survival. Although a small but statistically significant excess of renal tubular adenocarcinomas in high-dose males compared to vehicle-control males was calculated by statistical methods which adjusted for mortality, the NTP and its Board of Scientific Counselors have concluded that this study in Fischer 344 rats is inadequate for a judgment on the carcinogenicity of TCI. The following is quoted from the latest version (received May 24, 1983) of the abstract on this study from the NTP:

> Under the conditions of these studies, epichlorohydrin-free trichloroethylene caused renal tubular-cell tumors in male F344/N rats, produced nephropathy in both sexes, shortened the survival times of males and did not produce a carcinogenic response among females. This experiment was considered to be inadequate to evaluate the presence or absence of a carcinogenic response to trichloroethylene in male F344/N rats.

Use of additional lower doses in this study could have provided a broader evaluation of dose-response for the observed toxic effects of TCI.

8.1.1.2 National Cancer Institute (NCI 1976)--A carcinogenicity study of TCI in Osborne-Mendel rats was reported by the National Cancer Institute (NCI) in 1976. The composition of the TCI sample used, determined by vapor-phase chromatography-mass spectrometry, was as follows: TCI, $\geq$ 99.0% (w/w); 1,2-epoxybutane, 0.19%; ethyl acetate, 0.04%; N-methylpyrrole, 0.02%; diisobutylene, 0.03%; epichlorohydrin, 0.09%. The rats were 48 days old when the study began. Treatment groups included 50 animals/sex/dose, and 20 animals/sex comprised matched vehicle-control (corn oil) groups. Ninety-nine male and 98 female rats used as vehicle "colony controls" were also compared with treatment groups in this study. Vehicle "colony controls" included matched controls for TCI and other chemicals being tested concurrently.

Maximum and one-half maximum tolerated doses for the carcinogenicity study were estimated from survival, body weight, and necropsy results from a preliminary 8-week subchronic test. In the carcinogenicity study, treated male and female rats were given time-weighted average doses, by gavage, of 549 and 1,097 mg/kg/treatment of TCI 5 days/week for 78 weeks. Doses were presented as time-weighted averages due to the dose level changes made during the study, as shown in Table 8-3, on the basis of apparent toxicity from treatment with TCI.

Rats were allowed to survive until terminal sacrifice at 32 weeks post-treatment. Animals were observed daily for mortality and toxic signs, and body weights and food consumption were recorded weekly for the first 10 weeks and monthly thereafter. Survivors and decedents were necropsied, and tissues and organs, including tumors and lesions, were examined histopathologically.

At the beginning of the study, rats were randomly assigned to treatment and control groups according to body weight. Body weight trends in Tables 8-4 and 8-5 show decreased body weight gain in males and females in both treatment groups compared to matched vehicle-controls. Food consumption trends in Tables 8-4 and 8-5 indicate decreased food intake by both groups of treated female rats as compared to matched vehicle-controls, whereas food intake by treated male rats appeared comparable to that of matched vehicle-controls. Although decreased body weights in treated animals could reflect decreased food consumption, it would appear that decreases in food consumption and body weights ultimately were a result of toxicity from treatment with TCI. For example, food consumption between matched control and low-dose males was approximately equal, whereas body weight gain was less in the latter group.

TABLE 8-3. DOSAGE AND OBSERVATION SCHEDULE - TRICHLOROETHYLENE CHRONIC STUDY ON OSBORNE-MENDEL RATS (NTP 1982)

Dosage group	Dose (mg TCI/kg body wt)	Percent of TCI in corn oil	Age at dosing[a] (weeks)	Treatment period (weeks)	Time-weighted av. dose[b] (mg TCI/kg body wt)
Low-dose males and females	650	60.0	7	7[c]	
	750	60.0	14	9[c]	
	500	60.0	23	14[c]	
	500	60.0	37	48[d]	
	no treatment		85	32	549
High-dose males and females	1300	60.0	7	7[c]	
	1500	60.0	14	9[c]	
	1000	60.0	23	14[c]	
	1000	60.0	37	48[d]	
	no treatment	60.0	85	32	1097

Matched controls received doses of corn oil by gavage calculated on the basis of the factor for the high dose animals.

[a] Age at initial dose or dose change.

[b] Time-weighted average dose = Σ (dose in mg/kg x no. of days at that dose)/ Σ (no. of days receiving any dose). In calculating the time-weighted average dose, only the days an animal received a dose are considered.

[c] Dosing 5 days per week each week.

[d] Dosing 5 days per week, cycle of 1 week of no treatment followed by 4 weeks of treatment. (Animals were treated for 38 weeks of the 48-week period.)

Survival patterns in Figures 8-3 and 8-4 indicate a rather poor survival of animals in this study, particularly in treated animals where a decline in survival was evident early in the study. A dose-related decrease in survival of treated male rats was observed; however, survival of low-dose females was less than that of high-dose females early in the study. Statistical analysis of survival patterns in the NCI (1976) report showed a significant ($P < 0.05$) decrease in the survival of high-dose males ($P = 0.001$) vs. matched control

TABLE 8-4. MEAN BODY WEIGHTS, FOOD CONSUMPTION, AND SURVIVAL - TRICHLOROETHYLENE CHRONIC STUDY - MALE RATS (NCI 1976)

	Vehicle-control				Low dose				High dose			
	Body weight[a]				Body weight				Body weight			
Time interval (weeks)	Mean (g)	Std. dev. (g)	Food[b] (g)	No. of animals weighed	Mean (g)	Std. dev. (g)	Food (g)	No. of animals weighed	Mean (g)	Std. dev. (g)	Food (g)	No. of animals weighed
0	193	15.0	0	20	193	15.8	0	50	194	16.7	0	50
14	504	39.4	153	20	474	40.4	150	50	446	45.3	140	45
26	570	43.3	156	20	533	47.1	158	47	500	47.2	150	43
54	616	50.2	149	20	573	47.6	147	40	513	48.3	134	30
78	559	76.7	153	16	523	67.3	158	31	462	47.2	148	12
110	382	26.9	91	2	383	101.5	114	8	423	45.3	255	3

[a] Calculated using individual animal weight.
[b] Average weight per animal per week.

TABLE 8-5. MEAN BODY WEIGHTS, FOOD CONSUMPTION, AND SURVIVAL - TRICHLOROETHYLENE CHRONIC STUDY - FEMALE RATS (NCI 1976)

	Vehicle-control				Low dose				High dose			
Time interval (weeks)	Body weight[a] Mean (g)	Body weight[a] Std. dev. (g)	Food[b] (g)	No. of animals weighed	Body weight Mean (g)	Body weight Std. dev. (g)	Food (g)	No. of animals weighed	Body weight Mean (g)	Body weight Std. dev. (g)	Food (g)	No. of animals weighed
0	146	11.4	0	20	144	11.0	0	50	144	9.5	0	50
14	302	33.8	107	20	271	24.4	98	44	262	26.3	104	47
26	351	37.6	124	19	293	28.8	110	34	286	30.8	106	45
54	388	51.3	128	17	315	29.8	120	28	307	38.6	113	37
78	373	58.2	146	16	317	39.9	145	20	317	43.5	135	23
110	326	80.1	119	8	311	86.0	114	13	311	67.7	136	13

[a] Calculated using individual animal weight.
[b] Average weight per animal per week.

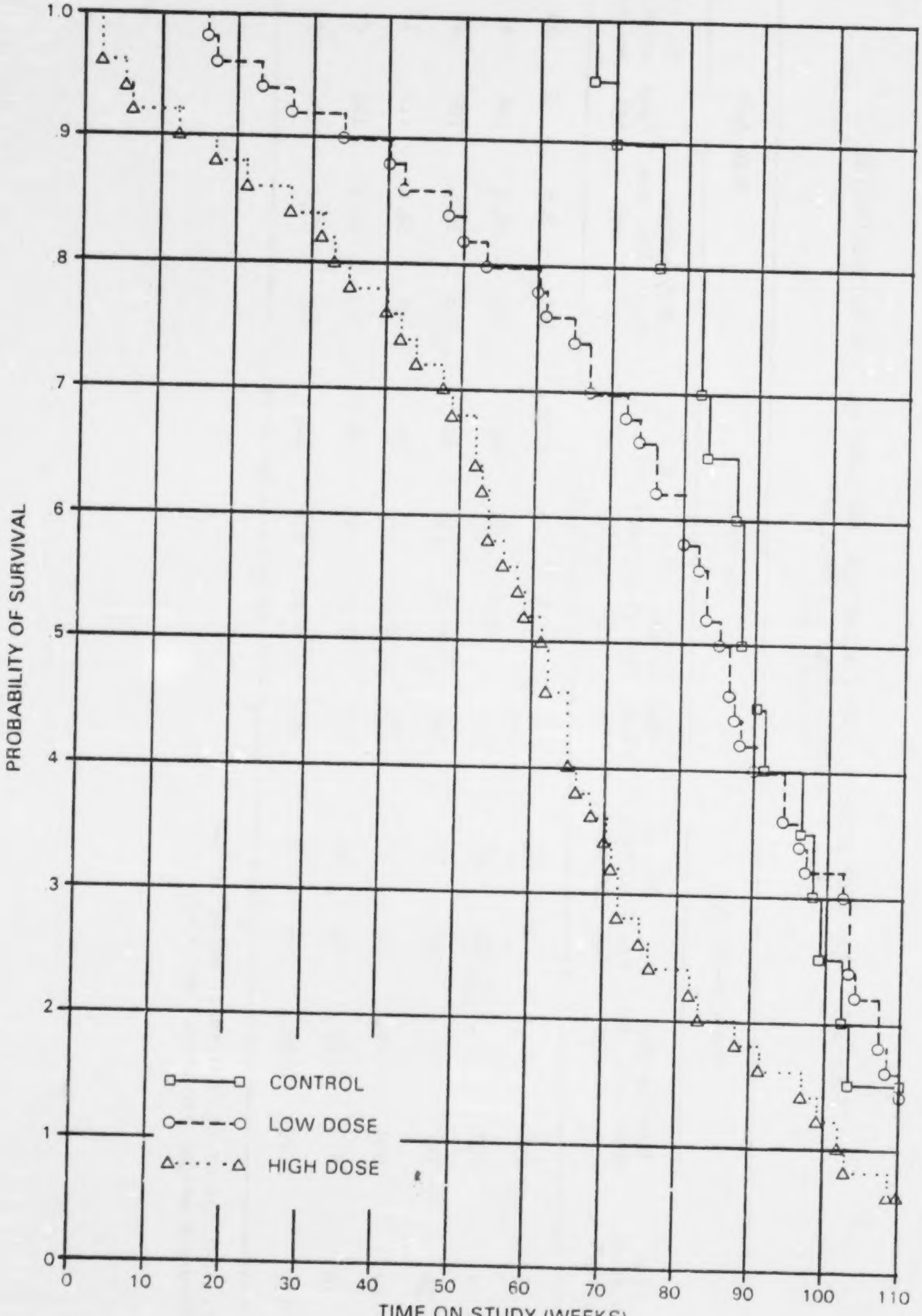


Figure 8-3. Product-limit estimates of probability of survival - trichloroethylene-treated male rats. (NCI 1976)

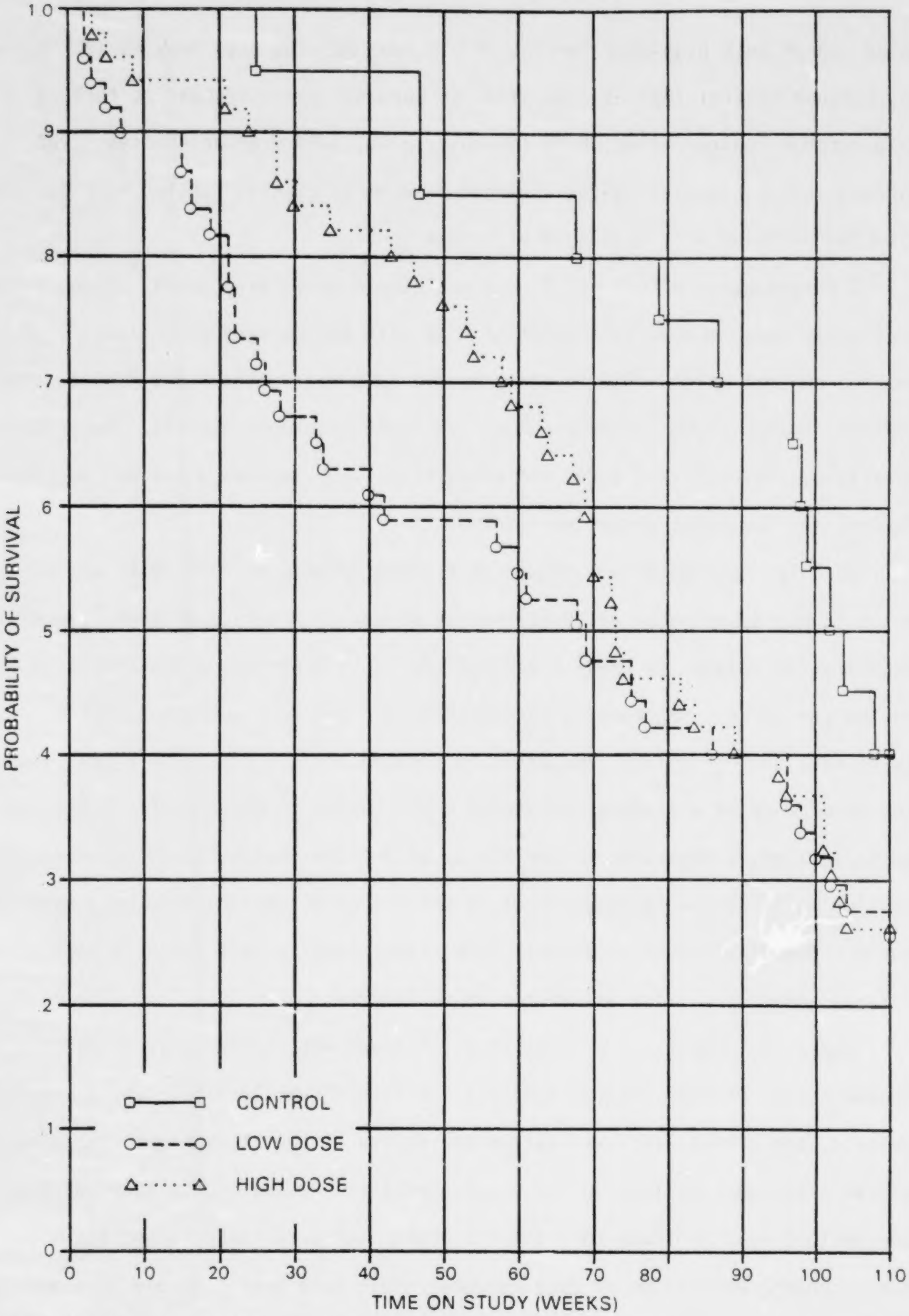


Figure 8-4. Product-limit estimates of probability of survival - trichloroethylene-treated female rats. (NCI 1976)

males and of both high-dose females ($P = 0.028$) and low-dose females ($P = 0.049$) vs. matched control females. Survival at terminal sacrifice was as follows: 3/20 matched control males, 8/50 low-dose males, 3/50 high-dose males, 8/20 matched control females, 15/48 low-dose females (2 missing females were excluded from the denominator), and 13/50 high-dose females.

A carcinogenic effect of TCI was not discerned in this study. Total tumor incidences expressed as the number of rats with tumors were as follows: 5/20 matched control males, 7/50 low-dose males, 5/50 high-dose males, 7/20 matched control females, 2/48 low-dose males, and 12/50 high-dose females. No statistically significant ($P < 0.05$) differences in tumor incidence between matched control and treatment groups were found.

Chronic nephropathy was common in treated animals of both sexes and both doses. This nephropathy was described as slight to moderate degenerative and regenerative changes in tubular epithelium. This nephropathy was stated to be unlike the chronic nephropathy encountered as a commonly occurring lesion in aging rats and recognized frequently in treated and control rats in this study. The occurrence of unilateral malignant mixed tumors in kidneys of two matched control males, a hamartoma in the kidney of one low-dose male and one matched control male, and an adenocarcinoma in the kidney of one low-dose male were noted. High incidences of chronic respiratory disease were found in each treated group as well as in matched controls.

Under the conditions of this study, TCI was not carcinogenic in Osborne-Mendel rats. However, animal survival was rather low, particularly in treated animals, and greater survival during the course of the study might have permitted a stronger evaluation for carcinogenicity. Reduction in survival and body weight gain indicate that the TCI doses used were toxic to the animals, and a broader evaluation of dose response could have been possible if additional lower doses had been tested and if dose levels had not been adjusted during the study. Rats treated with TCI, and their controls, were housed in a room with other rats treated with dibromochloropropane, ethylene dichloride, 1,1-dichloroethane, or carbon disulfide. There were 10 to 15 air exchanges each hour in the room, but ambient air was not tested for the presence of volatile materials.

8.1.1.3 Maltoni (1979)--Maltoni (1979) summarized the results of a study done to estimate the carcinogenic potential of TCI in Sprague-Dawley rats. Thirty males and 30 females, 13 weeks old, were assigned to each group. Two treatment groups received 50 or 250 mg/kg of TCI by gavage daily, 4 to 5 days weekly for 52 weeks, and a vehicle-control group was given olive oil. The TCI sample was at least 99.9% pure and was contaminated with $\leq$ 50 ppm each of 1,2-dichloroethylene, chloroform, carbon tetrachloride, and 1,1,2-trichloroethane. No detectable epoxides were found in this test material, and 10 ppm diisopropylamine was added as a stabilizer. Surviving animals were sacrificed at 140 weeks. All animals were given a necropsy examination, and tissues and organs were evaluated microscopically.

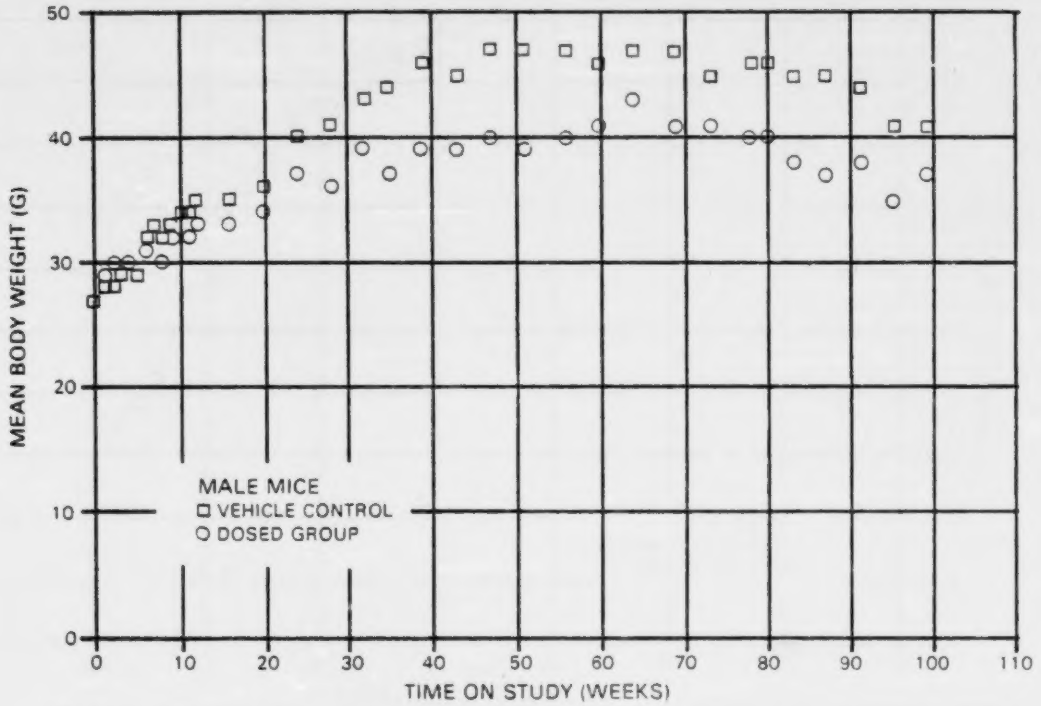
A carcinogenic effect was not demonstrated in this study. However, although the experiment lasted 140 weeks, the animals were dosed for 52 weeks, which is a duration below potential lifetime exposures. Tumor findings were presented in the study report; however, mortality patterns and toxic signs, if any, were not described to indicate the sensitivity of the animals to the TCI treatment used. Use of animals younger than 13 weeks of age at the beginning of the study could have given a broader assessment of the carcinogenic potential of TCI through an evaluation of treatment with TCI during the early growth and development of the rats.

8.1.2 Oral Administration (Gavage): Mouse

8.1.2.1 National Toxicology Program (NTP 1982)--A carcinogenicity bioassay on TCI in B6C3F1 mice was recently completed for the National Toxicology Program, and a 1982 draft report of the study has been reviewed by the NTP's Board of Scientific Counselors.

The TCI test sample and the experimental design were those used in the NTP (1982) carcinogenicity bioassay in Fischer 344 rats previously described in this document. Evaluation of mortality, body weights, and pathology in a 13-week preliminary dose-finding study led to the selection of 1,000 mg/kg/day, 5 days per week, for 103 weeks as the single dose level used in the bioassay. Fifty males and 50 females per group were assigned to untreated control groups, corn oil vehicle-control groups, and treatment groups, when 8 weeks old. Vehicle-control and treated animals received 0.5 ml corn oil with each gavage administration. Mice were caged in groups of 10 for the first 8 months of the study, and in groups of 5 for the rest of the study. The animals were evaluated for mortality, body weight, and pathology until terminal sacrifice at 112 to 115 weeks.

Results of this study were analyzed by the statistical methods used for the NTP (1982) carcinogenicity bioassay in rats. Mean body weights of treated males were lower than those of vehicle control males, whereas mean body weights between vehicle control and treated females were comparable (Figure 8-5). Survival was significantly ($P = 0.004$) lower in treated males compared to vehicle-controls, and although survival in treated females was lower after 95 weeks, the overall difference in survival between vehicle-control and treated females was not significant ($P < 0.05$) (Figure 8-6). Deaths of two vehicle-control males, three treated males, one vehicle-control female, and three treated females were attributed to "gavage error," and these animals were censored from the survival



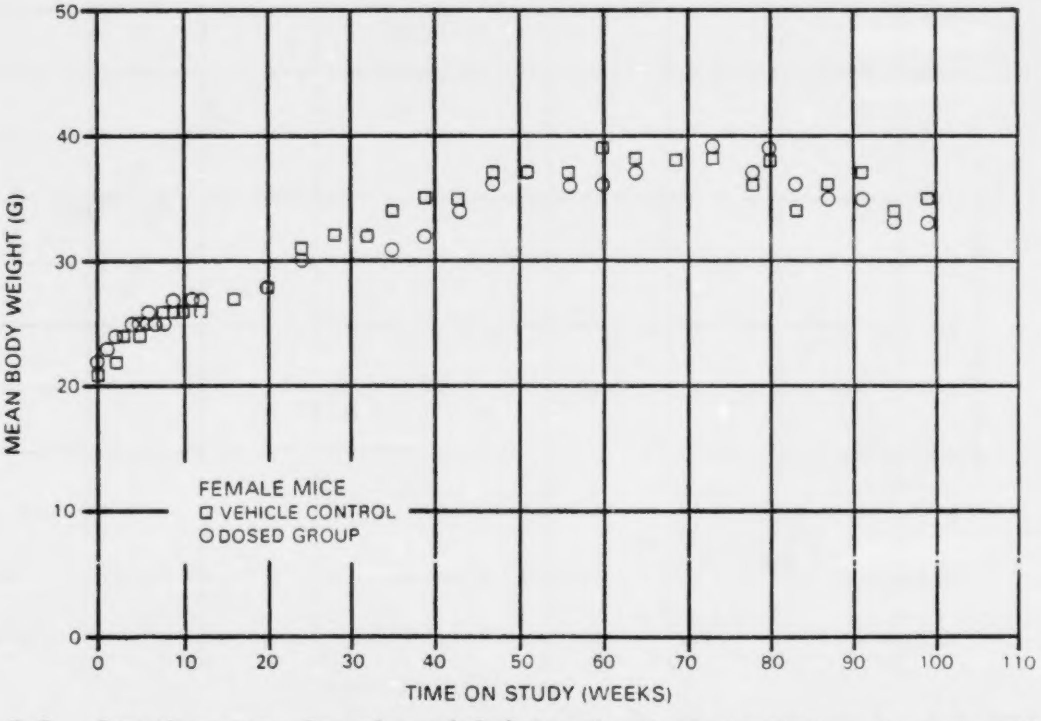


Figure 8-5. Growth curves for mice administered trichloroethylene in corn oil by gavage. (NTP 1982)

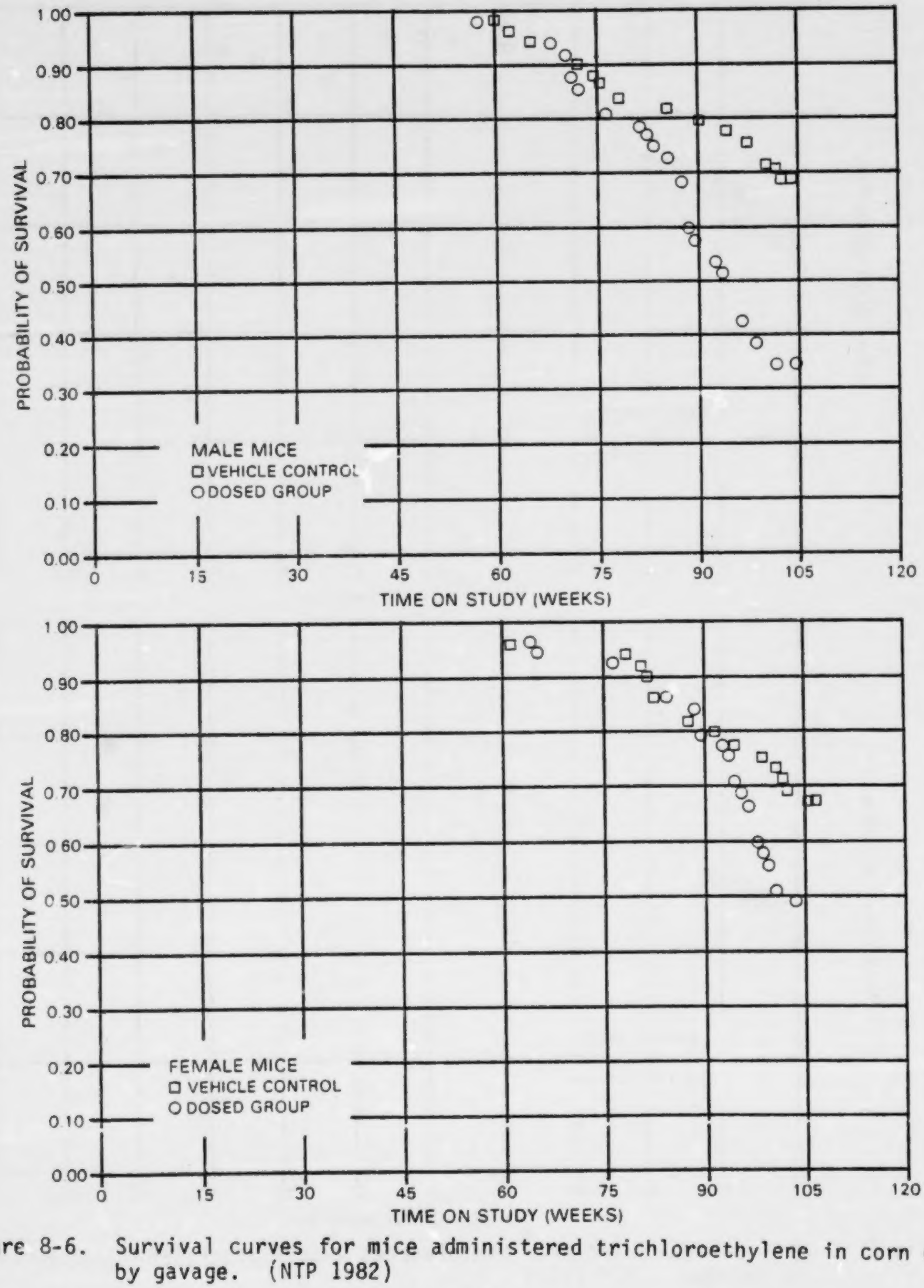


Figure 8-6. Survival curves for mice administered trichloroethylene in corn oil by gavage. (NTP 1982)

patterns shown in Figure 8-6. At terminal sacrifice, 33/50 vehicle-control males, 16/50 treated males, 32/50 vehicle-control females, and 23/50 treated females were alive.

A carcinogenic effect of TCI was not apparent in this study except for a significant ($P \leq 0.002$) increase in the incidence of hepatocellular carcinomas in treated male and female mice compared to corresponding vehicle-controls (Tables 8-6 and 8-7). Hepatocellular carcinomas metastasized to the lung in five treated males and one vehicle-control male. Hepatocellular adenoma incidence was significantly ($P < 0.05$) increased in treated female mice by each statistical method indicated in Table 8-7, but in treated male mice only by life table analysis (Table 8-6). Spontaneous hepatocellular tumor formation is a characteristic of the B6C3F1 mouse strain, and incidences of hepatocellular carcinoma in historical corn oil control B6C3F1 mice as of June 15, 1981 for carcinogenicity studies of 104 weeks duration in the NTP bioassay program were 18% (120/256) in males and 2.9% (22/751) in females.

Hepatocellular carcinomas had a general histopathologic appearance of markedly abnormal cytology and architecture, with diffuse patterns of tumor tissue infiltrating normal tissue, whereas hepatocellular adenomas consisted of circumscribed areas of distinct parenchyma cells. A treatment-related effect on nonneoplastic pathology in liver was not evident in the study report.

Cytomegaly in the kidney was found in 90% of treated male and 98% of treated female mice, but not in corresponding vehicle-controls. The average grades of severity for cytomegaly, according to the scheme used for grading cytomegaly in kidneys of treated Fischer 344 rats in the previously described NTP (1982) study, were 1.5 in dosed males and 1.8 in dosed females. Comparison with the average grades for male and female Fischer 344 rats given 1,000 mg/kg/day of TCI in the NTP (1982) bioassay indicates that toxic nephrosis of the kidney

TABLE 8-6. ANALYSIS OF PRIMARY TUMORS IN MALE MICE[a] (NTP 1982)

LIVER: ADENOMA

Tumor rates	Vehicle-control	Dosed
Overall[b]	3/48 (6%)	8/50 (16%)
Adjusted[c]	9.1%	27.5%
Terminal[d]	3/33 (9%)	1/16 (6%)
Statistical tests[e]		
Life table		P = 0.022
Incidental tumor test		P = 0.191
Cochran-Armitage Trend, Fisher Exact Tests		P = 0.113

LIVER: CARCINOMA

Tumor rates	Vehicle-control	Dosed
Overall[b]	8/48 (17%)	30/50 (60%)
Adjusted[c]	22.1%	92.5%
Terminal[d]	6/33 (18%)	14/16 (18%)
Statistical tests[e]		
Life table		P < 0.001
Incidental tumor test		P < 0.001
Cochran-Armitage Trend, Fisher Exact Tests		P < 0.001

LIVER: ADENOMA OR CARCINOMA

Tumor rates	Vehicle-control	Dosed
Overall[b]	11/48 (23%)	38/50 (76%)
Adjusted[c]	30.7%	97.1%
Terminal[d]	9/33 (27%)	15/16 (94%)
Statistical tests[e]		
Life table		P < 0.001
Incidental tumor test		P < 0.001
Cochran-Armitage Trend, Fisher Exact Tests		P < 0.001

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[a] The dosed group received doses of 1,000 mg/kg of trichlorethylene by gavage.

[b] Number of tumor-bearing animals/number of animals examined at the site.

[c] Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.

[d] Observed tumor incidence at terminal kill.

[e] Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Fisher Exact Test compares the overall incidence rates directly.

TABLE 8-7. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE[a] (NTP 1982)

LIVER: ADENOMA

Tumor rates	Vehicle-control	Dosed
Overall[b]	2/48 (4%)	8/49 (16%)
Adjusted[c]	6.2%	30.2%
Terminal[d]	2/32 (6%)	6/23 (26%)
Statistical tests[e]		
Life table		P = 0.016
Incidental tumor test		P = 0.023
Cochran-Armitage Trend, Fisher Exact Tests		P = 0.049

LIVER: CARCINOMA

Tumor rates	Vehicle-control	Dose
Overall[b]	2/48 (4%)	13/49 (27%)
Adjusted[c]	6.2%	43.9%
Terminal[d]	2/32 (6%)	8/23 (35%)
Statistical tests[e]		
Life table		P < 0.001
Incidental tumor test		P = 0.002
Cochran-Armitage Trend, Fisher Exact Tests		P = 0.002

LIVER: ADENOMA OR CARCINOMA

Tumor rates	Vehicle-control	Dosed
Overall[b]	4/48 (8%)	19/49 (39%)
Adjusted[c]	12.5%	63.8%
Terminal[d]	4/32 (13%)	13/23 (57%)
Statistical tests[e]		
Life table		P < 0.001
Incidental tumor test		P < 0.001
Cochran-Armitage Trend, Fisner Exact Tests		P < 0.001

---

[a] The dosed group received doses of 1,000 mg/kg of trichlorethylene by gavage.

[b] Number of tumor-bearing animals/number of animals examined at the site.

[c] Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.

[d] Observed tumor incidence at terminal kill.

[e] Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Fisher Exact Test compares the overall incidence rates directly.

was less severe in the mice in this study at an equivalent dose. Only one dosed mouse had a cytomegaly grade above 2. One vehicle-control male and one treated male each had a renal tubular cell adenocarcinoma.

Under the conditions of this bioassay, TCI treatment produced statistically significant increases of hepatocellular carcinomas in male and female B6C3F1 mice. These results repeated those of the NCI (1976) carcinogenicity study discussed herein, showing statistically significant increases in the incidence of hepatocellular carcinomas in male and female B6C3F1 mice given TCI stabilized with epoxides. In effect, the repeat study excludes the epoxides as necessary factors in the increased incidence of hepatocellular carcinomas in male and female B6C3F1 mice treated with TCI under the conditions of the NTP (1982) and NCI (1976) bioassays. Use of additional doses in the NTP (1982) study could have provided an evaluation of dose response for hepatocellular carcinomas in mice.

8.1.2.2 National Cancer Institute (NCI 1976)--A carcinogenicity study of TCI in B6C3F1 mice was reported by the National Cancer Institute (NCI) in 1976. The TCI sample was that used in the NCI (1976) study in Osborne-Mendel rats described herein. Treatment groups included 50 animals/sex/dose, and 20 animals/sex comprised matched vehicle-control (corn oil) groups. Seventy-seven male and 80 female mice used as vehicle "colony controls" and 70 male and 76 female mice serving as untreated "colony controls" were also compared with the treatment groups in this study; "colony controls" included matched controls for TCI and other chemicals being tested concurrently. Mice were 35 days old when the study began.

Maximum and one-half maximum tolerated doses for the carcinogenicity study were estimated from survival, body weight, and necropsy results from a preliminary 8-week subchronic test. In the carcinogenicity study, time-weighted average doses of TCI were given by gavage, 5 days/week, for 78 weeks, to the mice at levels of 1,169 and 2,339 mg/kg/treatment in males and 869 and 1,739 mg/kg/treatment in females. Doses were presented as time-weighted averages due to the dose level changes made during the study, as shown in Table 8-8, in an attempt to maintain maximum and one-half maximum tolerated doses.

TABLE 8-8. DOSAGE AND OBSERVATION SCHEDULE - TRICHLOROETHYLENE CHRONIC STUDY ON B6C3F1 MICE (NCI 1976)

Dosage group	Dose (mg TCI/kg body wt)	Percent of TCI in corn oil	Age at dosing[a] (weeks)	Treatment period (weeks)	Time-weighted Av. dose[b] (mg TCI/kg body wt)
Low-dose males	1000	15.0	5	6[c]	
	1000	10.0	11	6[c]	
	1200	24.0	17	66[c]	
	no treatment		83	12	1169
High-dose males	2000	15.0	5	6[c]	
	2000	20.0	11	6[c]	
	2400	24.0	17	66[c]	
	no treatment		83	12	2339
Low-dose females	700	10.0	5	12[c]	
	900	18.0	17	66[c]	
	no treatment		83	12	869
High-dose females	1400	10.0	5	6[c]	
	1400	20.0	11	6[c]	
	1800	18.0	17	66[c]	
	no treatment		83	12	1739

Matched controls received doses of corn oil by gavage calculated on the basis of the factor for the high dose animals.

[a] Age at initial dose or dose change.

[b] Time-weighted average dose = Σ (dose in mg/kg x no. of days at that dose)/Σ (no. of days receiving any dose). In calculating the time-weighted average dose, only the days an animal received a dose are considered.

[c] Dosing 5 days per week each week.

Mice were permitted to survive until terminal sacrifice at 12 weeks following treatment. Animals were observed daily for mortality and toxic signs, and body weights and food consumption were recorded weekly for the first 10 weeks and monthly thereafter. Survivors and decedents were necropsied, and tissues and organs, including tumors and lesions, were examined histopathologically.

At the beginning of the study, mice were randomly assigned to treatment and control groups according to body weight. Body weight trends in Tables 8-9 and 8-10 show comparable weight gain between matched vehicle-controls and both treatment groups for both males and females. Food consumption trends in Tables 8-9 and 8-10 indicate similar consumption between the female groups and slightly higher consumption in treated males as compared to matched controls. Therefore, it is possible that, if food consumption between matched control and treated groups of males had been equivalent, body weight gain in treated males might have been less than that in matched control males.

Survival patterns are presented in Figures 8-7 and 8-8. Differences in survival reported as statistically significant ($P < 0.05$) were those between high-dose and low-dose males ($P = 0.001$), matched control males and low-dose males ($P = 0.004$), and both treatment groups of females and matched control females ($P = 0.035$). Survival at terminal sacrifice was as follows: 8/20 matched control males, 36/50 low-dose males, 22/50 high-dose males, 20/20 matched control females, 42/50 low-dose females, and 42/47 high-dose females (3 high-dose females were missing and not included in the denominator).

Prior to terminal sacrifice, approximately 50% of the treated males and "a few" treated females exhibited bloating or abdominal distension. Results of necropsy and histopathologic examinations revealed a statistically significant ($P < 0.05$) increase in the incidence of hepatocellular carcinomas in male and

TABLE 8-9. MEAN BODY WEIGHTS, FOOD CONSUMPTION, AND SURVIVAL - TRICHLOROETHYLENE CHRONIC STUDY - MALE MICE (NCI 1976)

	Vehicle-controls				Low dose				High dose			
Time interval (weeks)	Body weight[a] Mean (g)	Body weight[a] Std. dev. (g)	Food[b] (g)	No. of animals weighed	Body weight Mean (g)	Body weight Std. dev. (g)	Food (g)	No. of animals weighed	Body weight Mean (g)	Body weight Std. dev. (g)	Food (g)	No. of animals weighed
0	17	0.5	0	20	17	2.0	0	50	17	1.1	0	50
14	28	0.1	23	20	28	0.8	27	50	29	0.4	26	49
26	31	0.2	24	20	31	0.9	28	48	32	0.7	27	42
54	32	0.4	21	18	33	1.0	26	45	34	0.4	30	33
78	34	0.6	24	8	34	0.8	32	40	35	1.0	39	24
90	34	0.7	34	8	33	0.7	32	35	34	1.3	38	20

[a] Calculated using individual animal weight.
[b] Average weight per animal per week.

TABLE 8-10. MEAN BODY WEIGHTS, FOOD CONSUMPTION, AND SURVIVAL - TRICHLOROETHYLENE CHRONIC STUDY - FEMALE MICE (NCI 1976)

Time interval (weeks)	Vehicle-controls				Low dose				High dose			
	Body weight[a] Mean (g)	Body weight[a] Std. dev. (g)	Food[b] (g)	No. of animals weighed	Body weight Mean (g)	Body weight Std. dev. (g)	Food (g)	No. of animals weighed	Body weight Mean (g)	Body weight Std. dev. (g)	Food (g)	No. of animals weighed
0	14	0.0	0	20	14	0.6	0	50	14	0.7	0	50
14	23	0.4	27	20	23	0.3	23	49	24	0.5	24	49
26	25	0.2	22	19	26	0.7	23	49	25	0.5	24	47
54	28	1.1	19	18	27	0.5	21	45	26	0.3	22	41
78	29	0.6	23	18	29	0.7	25	41	28	0.4	26	40
90	28	0.6	28	17	30	0.6	27	40	28	0.7	26	39

[a] Calculated using individual animal weight.
[b] Average weight per animal per week.

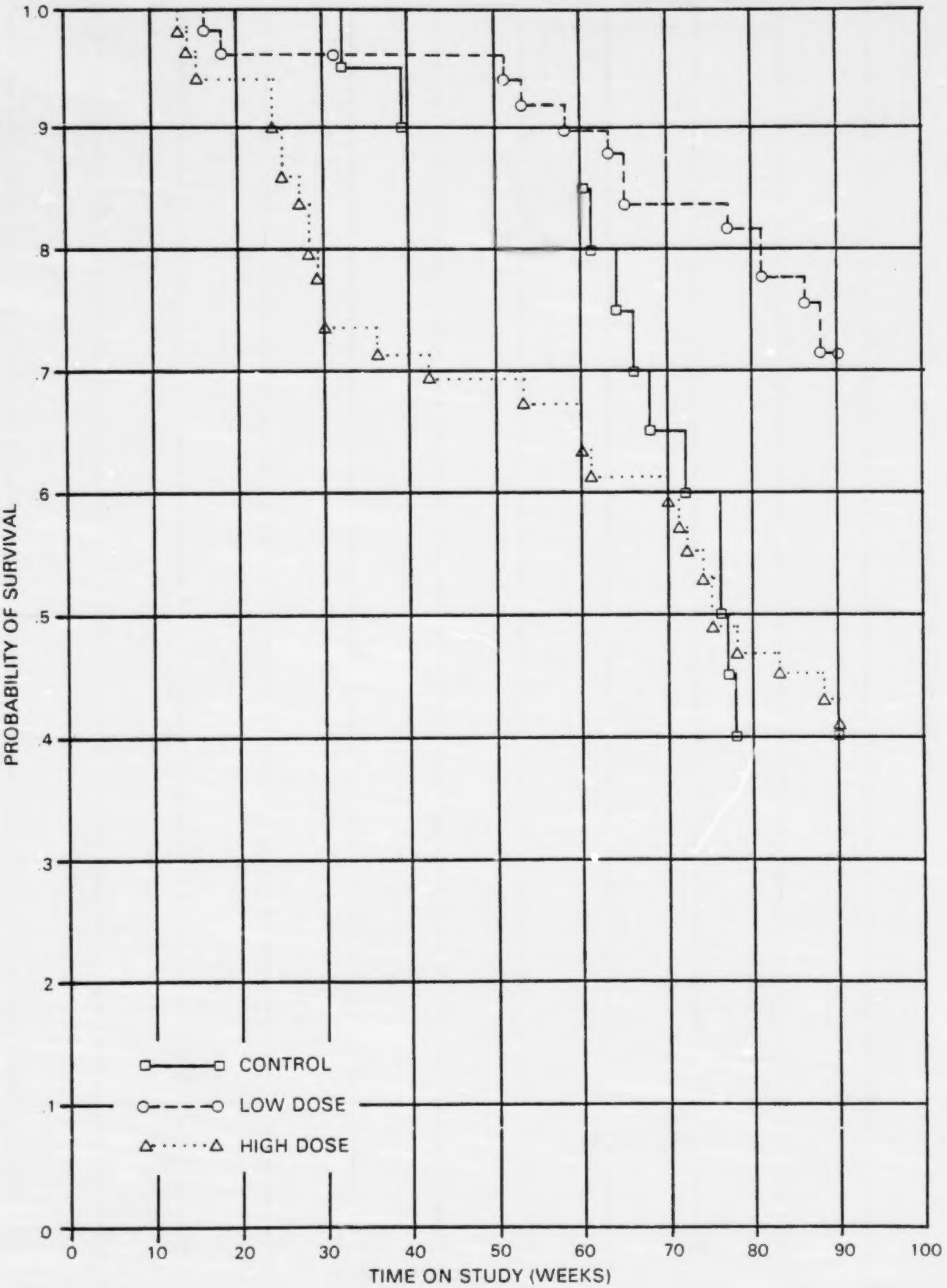

Figure 8-7. Product-limit estimates of probability of survival - trichloroethylene-treated male mice. (NCI 1976)

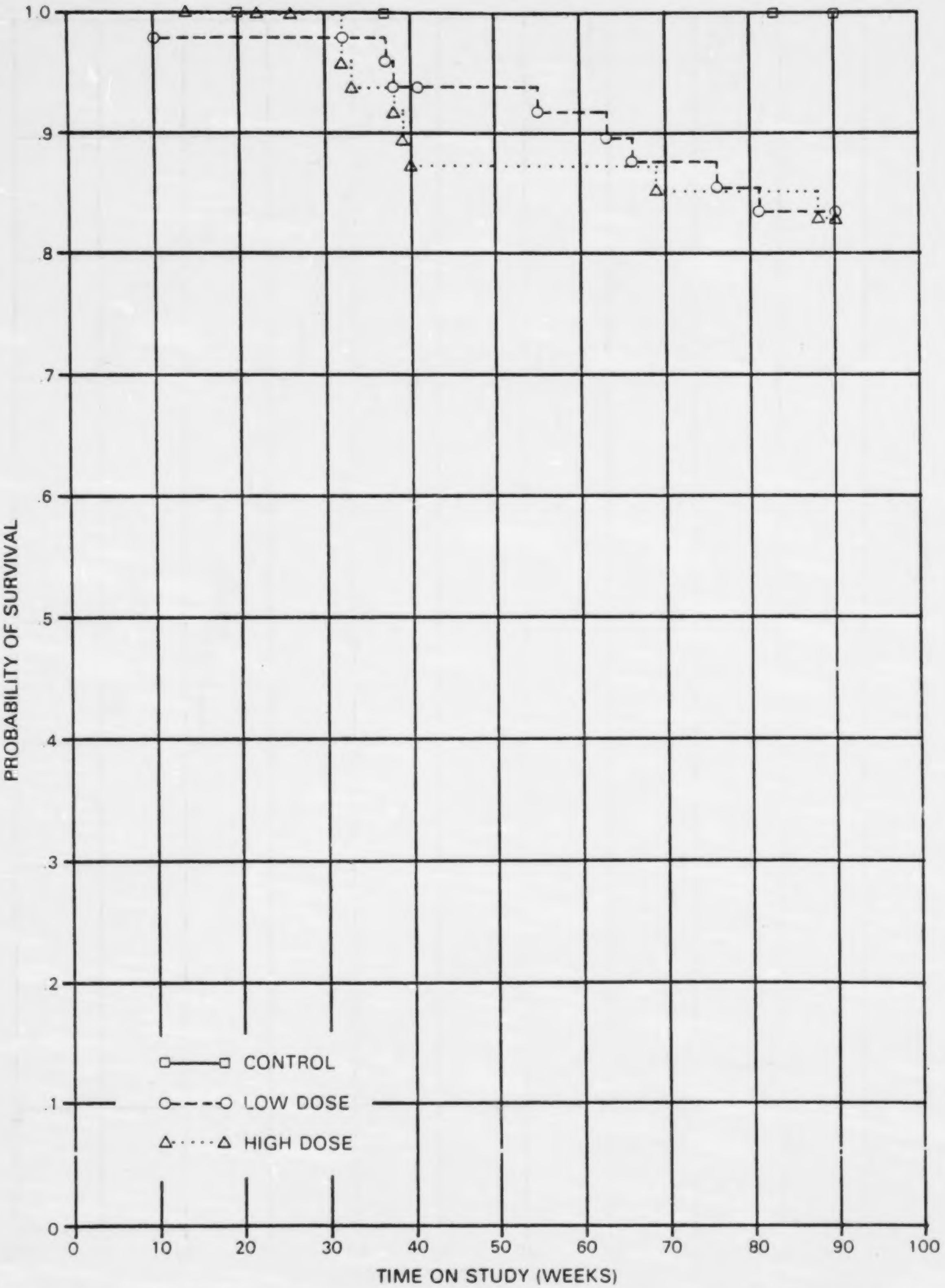

Figure 8-8. Product-limit estimates of probability of survival - trichloroethylene-treated female mice. (NCI 1976)

female mice treated with TCI (Table 8-11). A dose-related response in females is evident. Metastasis of hepatocellular carcinoma to the lung was observed in four low-dose males and in three high-dose males. The time to observation of the first hepatocellular carcinoma in each group was as follows: 72 weeks in matched control males, 81 weeks in low-dose males, 27 weeks in high-dose males, and 90 weeks in females. With regard to "colony control" mice, hepatocellular carcinomas were diagnosed in 5/77 (6.5%) vehicle-control males, 5/70 (7.1%) untreated control males, 1/80 (1.3%) vehicle-control females, and 2/76 (2.6%) untreated control females. Hepatocellular carcinoma incidences in "colony control" mice were comparable to those in matched vehicle-control males (1/20 or 5%) and females (0/20 or 0%). Statistically significant ($P < 0.05$) differences in the incidence of other tumor types between control and treatment groups were not found.

The general pathology of hepatocellular carcinomas consisted of various characteristics, including an orderly cord-like arrangement of neoplastic cells, a pseudoglandular pattern resembling adenocarcinoma, or sheets of highly anaplastic cells with minimal cord- or gland-like arrangement.

Under the conditions of this study, TCI induced a statistically significant ($P < 0.05$) increase in hepatocellular carcinoma incidence in male and female B6C3F1 mice. Although the number of matched vehicle controls was low, the use of pooled colony controls gives additional support for treatment-related effects.

A more precise estimate of dose response perhaps could have been obtained if additional lower doses had been used and if constant doses rather than time-weighted averages had been used. Treated animals were housed in the

TABLE 8-11. INCIDENCE OF HEPATOCELLULAR CARCINOMAS IN B6C3F1 MICE RECEIVING TRICHLOROETHYLENE BY ORAL GAVAGE (NCI 1976)

	Males			Females		
Dose	0 mg/kg	1169 mg/kg	2339 mg/kg	0 mg/kg	869 mg/kg	1739 mg/kg
Number with tumors/number of animals	1/20	26/50	31/48	0/20	4/50	11/47
Incidence	0.05	0.52	0.646	0	0.08	0.234
P-value		1.39×10^{-4}[a]	3.44×10^{-6}[a]		1.75×10^{-1}	1.35×10^{-2}[a]
Number with metastases	0/20	4/50	3/48	0/20	0/50	0/47

[a] Statistically significant ($P < 0.05$) increase compared to matched vehicle-controls (0 mg/kg) by Fisher Exact Test.

same room as mice treated with other volatile compounds*; however, since 1) controls were in the same room as treated animals, 2) oral TCI doses probably would have been much higher than ambient levels of other volatiles, 3) the cages had filters to limit the amount of chemical released into the ambient air, 4) the total room air was exchanged 10 to 15 times per hour, and 5) dosing was done in another room under a large hood, the likelihood that the other volatile compounds were responsible for the observed results is considered low. It should be noted that ambient levels of volatiles in the animal quarters were not measured. Further evidence that the induction of hepatocellular carcinomas in B6C3F1 mice was due to treatment with TCI in this study is the repeat study (NTP 1982) with a similar experimental design in which the mice were not housed with mice treated with other volatile chemicals. Additionally, the repeat NTP (1982) study of purified TCI in B6C3F1 mice devoid of detectable levels of epoxides indicates that the presence of epoxides was not necessary for the induction of hepatocellular carcinomas in B6C3F1 mice in the NCI (1976) study.

8.1.2.3 Van Duuren et al. (1979)†--A carcinogenicity study of purified TCI in ICR/Ha Swiss mice was described by Van Duuren et al. (1979). Experimental details provided below include those obtained through written communication with B.L. Van Duuren, New York University. The TCI sample was > 99% pure, and, although contaminants were not identified, epoxides were considered not to be present in the test material. It was stated that although range-finding studies were done to estimate maximum tolerated doses (doses which did not affect body weight gain or induce clinical signs of toxicity in a 6- to 8-week test which included histopathologic examination of treatment site sections) for all test groups in this study, TCI doses used in the main topical application experiments were below those maximally tolerated. TCI doses used in the skin treatment experiments were selected to permit a comparison with TCI oxide, with respect to carcinogenicity, on an equimolar basis. This study incorporated the following experiments: 1) 30 females received topical applications of 1 mg TCI three times weekly for approximately 581 days; 2) 30 females were treated topically with a single application of 1.0 mg TCI followed 14 days later by applications of 2.5 ug phorbol myristate acetate (PMA) to the skin three times weekly until termination of the study at 452 days. Thirty females similarly treated with 1.0 mg TCI epoxide and 2.5 ug PMA were also tested; 3) 30 females were given subcutaneous injections of 0.5 mg TCI once weekly for 622 days; and 4) 30 males and 30 females were administered 0.5 mg TCI by gavage once weekly for 622 days. A vehicle-control group of 30 mice and an untreated control group of 100 mice were included in each experiment. Dosing vehicles were acetone in the skin treatment tests and trioctanoin in the other tests. In the initiation-promotion experiment, 210 mice treated with PMA alone were also on test. The mice were 6 to 8 weeks old at the beginning of the study, and six animals were housed in each cage. Test sites on the skin were shaved as necessary and were not covered, and test samples were administered in ventilated hoods; however, it was the authors' impression that TCI was immediately absorbed and that evaporation from test sites was minimal. The animals were weighed monthly, and each animal was examined by necropsy. Tumors and lesions were examined histopathologically. Histopathologic evaluation was routinely done on skin, liver, stomach, and kidney in the skin application experiments, injection site tissue and liver in the subcutaneous injection experiment, and stomach, liver, and kidney in the gavage experiment.

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*1,1,2,2-tetrachloroethane, 3-chloropropene, chloropicrin, 1,1-dichloroethane, chloroform, sulfolene, iodoform, ethylene dichloride, methyl chloroform, 1,1,2-trichloroethane, tetrachloroethylene, hexachloroethane, carbon disulfide, trichlorofluoromethane, carbon tetrachloride, ethylene dibromide, dibromochloropropane.

†Although discussed under the heading of Oral Administration: Gavage (Mouse), this study in mice also includes subcutaneous injection, repeated skin application, and initiation-promotion experiments with TCI and an initiation-promotion experiment with TCI oxide.

Treatment with TCI and TCI oxide did not produce statistically significant ($P < 0.05$) increases in tumor incidence in this study (Tables 8-12, 8-13, and 8-14). In the initiation-promotion experiment, the numbers of mice with skin papillomas (squamous cell carcinomas) were: 4 (1) treated with TCI, 15 (3) treated with PMA alone, and none in the control groups. Repeated application to the skin produced lung and stomach tumors in 9 and 1 TCI-treated mice, respectively, 11 and 2 vehicle-treated controls, respectively, and 30 and 5 untreated controls, respectively. Lung tumors were diagnosed as benign papillomas, and stomach tumors were described as papillomas of the forestomach. Tumors were not found in TCI-treated and control animals in the experiment with subcutaneous doses. In the experiment with gavage dosing, forestomach tumors were observed in one female and two male mice given TCI, no vehicle-control animals, and five female and eight male (one male had a squamous cell carcinoma in the forestomach) untreated control mice. Three mice given repeated applications of TCI oxide onto the skin had skin papillomas. Results in Tables 8-12, 8-13, and 8-14 show that the mice were sensitive to positive control agents under the design of this study. Median survival times approximated the number of days the animals were on test, and body weights were comparable between treatment and control groups in each experiment.

Use of TCI doses as high as those maximally tolerated could have more strongly challenged the mice for carcinogenicity in the skin treatment experiments, but Van Duuren et al. (1983) noted that low activity or lack of activity of chloroolefins tested for carcinogenicity in skin treatment experiments in their laboratory was expected because of evidence for the relatively low level of epoxidizing enzymes in mouse skin relative to other organs such as liver.

TABLE 8-12. MOUSE SKIN BIOASSAYS OF TRICHLOROETHYLENE AND TRICHLOROETHYLENE OXIDE
(30 FEMALE HA:ICR SWISS MICE PER GROUP)
(Van Duuren et al. 1979)

	Initiation-promotion[a]			Repeated application[b]			
Compound	Dose, mg/ application/ mouse	Days to first tumor	Mice with papillomas/ total papillomas	Dose, mg/ application/ mouse	Days to first tumor	Mice with papillomas/ total papillomas	No. of mice with distant tumors[d]
Trichloroethylene	1.0	264	4/9 (1)[c]	1.0	-	0	9 lung 1 stomach
Trichloroethylene oxide	1.0	371	3/3	NT	-	-	-
7,12-Dimethyl-benz[a]anthracene[e]	0.02	54	29/317 (18) $P < 0.0005$[f]	NT	-	-	-
PMA controls							
(120 mice)	0.0025	141	9/10 (1)				
(90 mice)	0.005	449	6/7 (2)				
Acetone (0.1 ml)	-	-	-	0.1 ml	-	0	11 lung 2 stomach
No treatment (100 mice)	-	-	-	-	-	0	30 lung 5 stomach

[a] Compounds were applied to dorsal skin in 0.1 ml acetone once followed 14 days later with 2.5 mg PMA in 0.1 ml acetone 3 times weekly.

[b] Compounds applied in 0.1 ml acetone 3 times weekly. NT = Not tested.

[c] Number of mice with squamous cell carcinomas are in parenthesis.

[d] Lung tumors were benign papillomas, and stomach tumors were papillomas of the forestomach.

[e] Positive control agent.

[f] Statistically significant by chi-square test compared to controls.

TABLE 8-13. SUBCUTANEOUS INJECTION OF TRICHLOROETHYLENE
(30 FEMALE HA:ICR SWISS MICE PER GROUP)
(Van Duuren et al. 1979)

Compound and dose[a]	Days on test	No. of mice with local sarcomas[b]	P value[c]
Trichloroethylene, 0.5	622	0	NS
B-propiolactone,[d] 0.3	378	24	<0.0005
Trioctanoin, 0.05 ml	631	0	-
No treatment (100 mice)	649	0	-

[a] Compound subcutaneously injected in left flank once weekly in 0.05 ml trioctanoin. Dose is mg per injection per mouse.

[b] Histopathologically characterized as fibrosarcomas.

[c] NS = Not significant. Statistical significance calculated by chi-square test compared to controls.

[d] Positive control agent.

TABLE 8-14. CARCINOGENICITY OF TRICHLOROETHYLENE BY INTRAGASTRIC FEEDING IN MALE AND FEMALE HA:ICR SWISS MICE (30 MICE PER GROUP)

(Van Duuren et al. 1979)

Compound and dose[a]	Sex	Days on test	No. of mice with forestomach tumors[b]	P value[c]
Trichloroethylene, 0.5	M	622	1	NS
	F	622	2	NS
B-Propiolactone, 1.0	M	539	24(23)	<0.0005
	F	582	25(23)	<0.0005
Trioctanoin, 0.1 ml	M	631	0	-
	F	635	0	-
No treatment				
(100 mice)	M	649	5	-
(60 mice)	F	636	8(1)	-

[a] Dose in mg per intubation per mouse. Compounds were given once weekly in 0.1 ml trictanoin.

[b] Number of mice with squamous cell carcinoma of the forestomach is given in parentheses.

[c] NS = Not significant. Statistical significance calculated by chi-square test compared to controls.

Administration of TCI more than once each week in the experiments with oral and subcutaneous treatment also might have more strongly challenged the animals for carcinogenicity. Dr. Van Duuren noted (written communication) that once-weekly subcutaneous injection would allow time for the injection site to heal before subsequent injections; however, more than 1 injection per week might have more effectively tested the carcinogenic potential of TCI at sites distant from the injection site. Routine histopathologic examination of a greater range of tissues and organs might have provided a broader evaluation for carcinogenicity.

8.1.3 Inhalation Exposure: Rats

8.1.3.1 Henschler et al. (1980)--Henschler et al. (1980) described a study on the carcinogenic potential of TCI in Han:Wist rats. The initial age of the animals was not given. Thirty male and 30 female rats were assigned to each of three dose groups which were exposed to 0 (controls exposed to inhalation chamber air only), 100 (0.55 mg/l air), or 500 (2.8 mg/l air) ppm TCI vapor 6 hours daily, 5 days/week, for 18 months. The animals were individually caged in this study. Surviving rats were sacrificed at 36 months. All animals were subjected to necropsy and histopathologic examination.

The TCI sample, stabilized with 0.0015% triethanolamine, contained the following impurities by gas chromatography-mass spectrometry analysis: chloroform, carbon tetrachloride, 1,1,2-trichloroethane, 1,1,1-trichloroethane, and 1,2,2-tetrachloroethane, each present at less than 0.0000025% (w/w). Epoxides were not detected in this test material. Exposure levels of TCI in the inhalation chambers were monitored with direct ultraviolet spectrophotometry, and the flow rate of the TCI air mixture was adjusted to yield a threefold turnover of the total volume each hour. A description of the analytical method was provided by D. Henschler, University of Wurzburg, as follows: Exposure levels were automatically controlled to 100 or 500 ppm, as appropriate, and were continuously monitored in a gas-flow cell in an ultraviolet beam of 205 mm. The accuracy of this method was determined by the noise of the electronic equipment, and the registered band indicated ± 7% of the median at 100 ppm.

No effect of TCI on body weight gain was observed. A statistically significantly ($P < 0.05$) decrease in survival of treated rats was not found (Table 8-15).

A carcinogenic effect of TCI was not evident in this study. Use of a maximum tolerated dose is not indicated in the study report, and the comparability of survival and body weight between control and treatment groups indicates that higher exposure levels of TCI possibly could have been used to provide a broader evaluation of TCI carcinogenicity.

TABLE 8-15. ESTIMATION OF PROBABILITY OF SURVIVAL OF RATS (%)[a]
(Henschler et al. 1980)

	Group					
	Control		100 ppm		500 ppm	
Week	M	F	M	F	M	F
1	100.0	100.0	100.0	100.0	100.0	100.0
50	96.6	100.0	96.6	96.6	100.0	93.3
75	93.3	96.6	96.6	93.3	100.0	90.0
100	86.7	86.7	90.0	86.6	90.0	76.6
115	83.3	70.0	86.6	63.3	76.6	50.0
126	66.7	56.6	80.0	50.0	66.6	50.0
156	46.7	16.7	23.3	13.3	36.7	16.7

[a] By the Kaplan and Meier (1958) method.

8.1.3.2 Bell et al. (1978; audit of Industrial Bio-Test Laboratories, Inc. study)--A carcinogenicity study of TCI vapor in Charles River rats was conducted at Industrial Bio-Test Laboratories, Inc. (IBT) from 1975 to 1977, and audit findings by the Manufacturing Chemists Association (MCA) on this study have been reported (Bell et al. 1978). The TCI product used in this study contained, on a weight percent basis, TCI, > 99%; diisobutylene, 0.023%; butylene oxide, 0.24%; ethyl acetate, 0.052%; N-methylpyrrole, 0.008%; and epichlorohydrin, 0.148%. Each of the three treatment groups plus an untreated control group consisted of 120 rats/sex. Treated animals were exposed to nominal concentrations of 100, 300, or 600 ppm (0.55, 0.165, or 0.330 mg/l air) TCI vapor 6 hours/day, 5 days/week, for 24 months. Surviving animals were sacrificed on termination of treatment. Thirty rats/sex scheduled for interim sacrifice to evaluate hematology and clinical chemistry were not given pathologic examinations.

According to the audit report by the MCA, there were noticeable deficiencies in the performance of this study. Examination of analytical data on the TCI vapor concentrations in the inhalation chambers revealed wide deviations from the nominal concentrations and high variability in the number of measurements made daily which do not allow a precise determination of the actual exposure levels. For example, histograms of measurements (Figures 8-9, 8-10, and 8-11) made during the initial 120 days of exposure show a clustering of data around the nominal concentrations; however, only 259/565, 393/623, and 389/598 readings made during the first 6 months of the study fell within ± 30% of the 100, 300, and 600 ppm nominal concentrations, respectively. Exposure data in the report by Bell et al. (1978) cover the initial 6 months of this study, and additional information from the Chemical Manufacturers Association has been requested regarding exposure levels obtained during the final 18 months of this study.

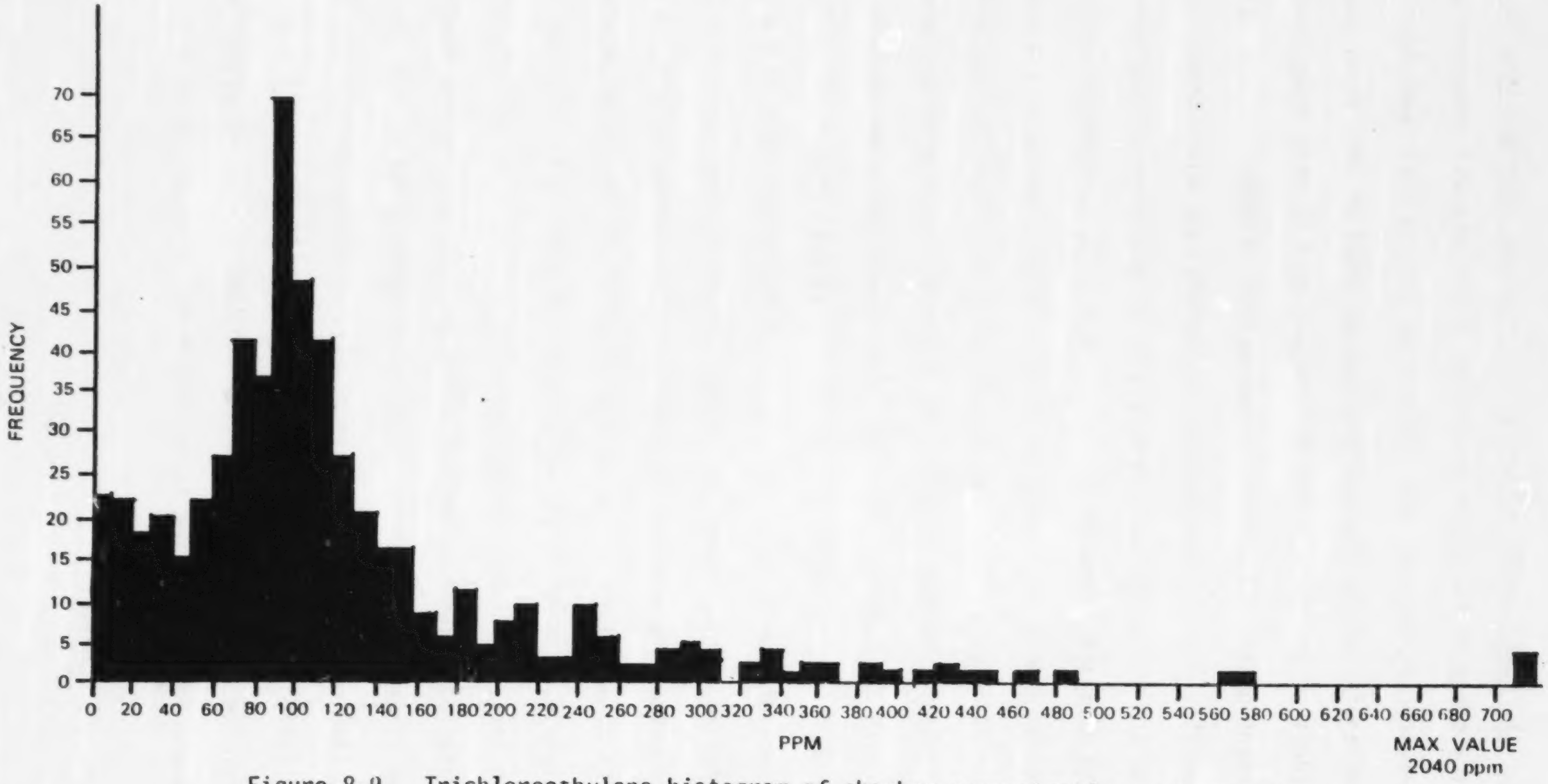

Figure 8-9. Trichloroethylene histogram of chamber concentration measurements T-1 (100 ppm). (Bell et al. 1978)

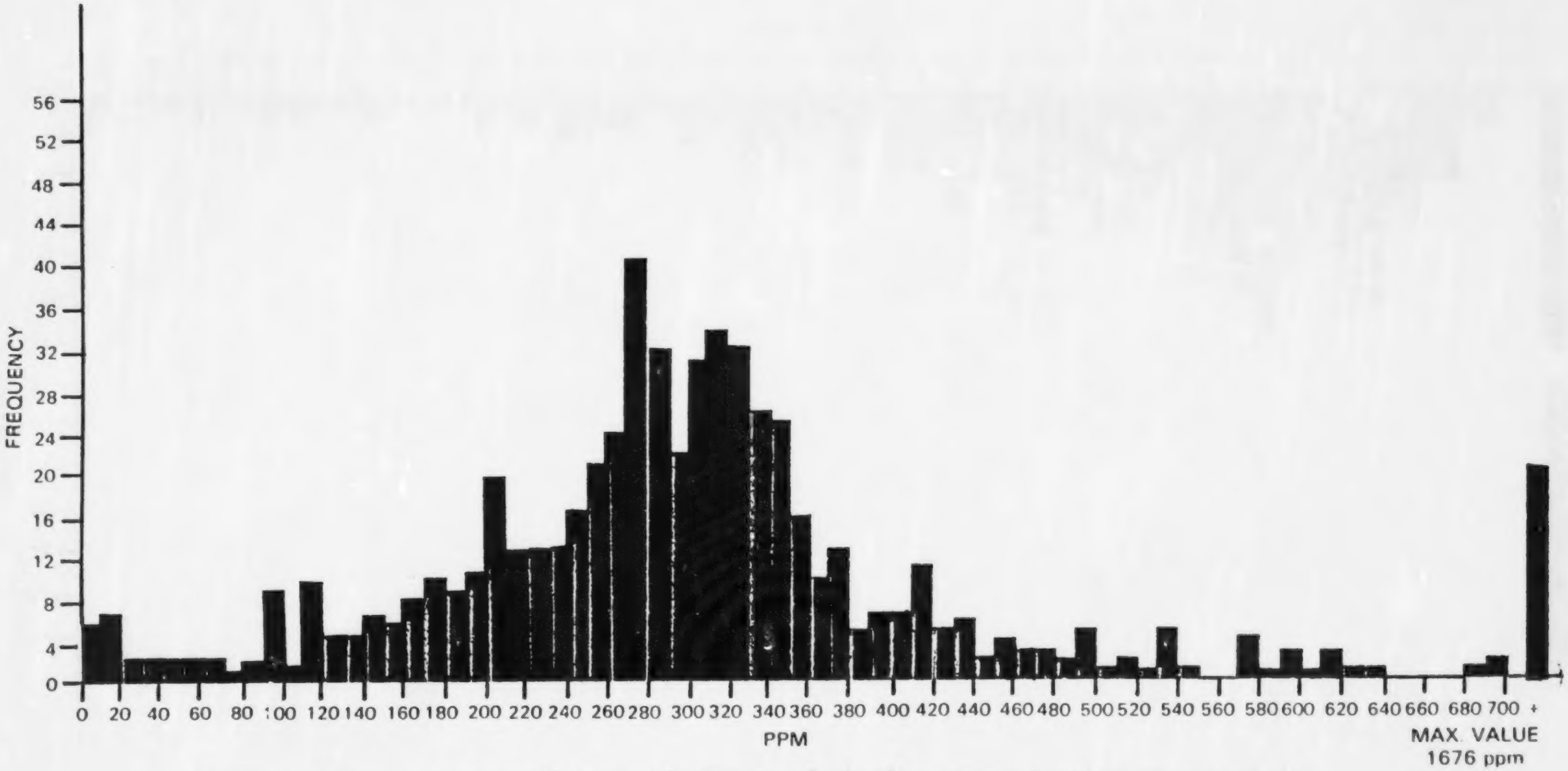

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Figure 8-10. Trichloroethylene histogram of chamber concentration measurements T-2 (300 ppm). (Bell et al. 1978)

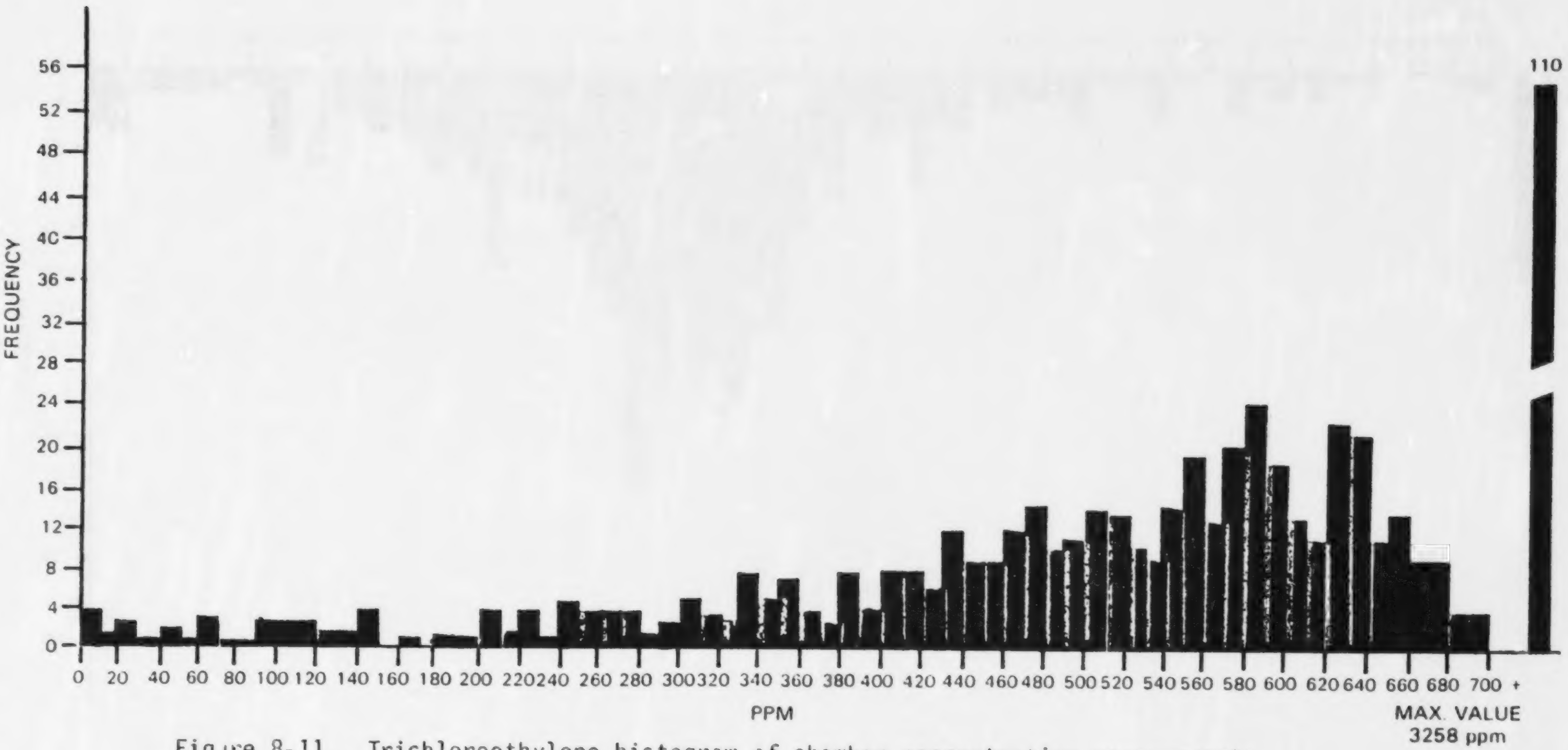

Figure 8-11. Trichloroethylene histogram of chamber concentration measurements T-3 (600 ppm). (Bell et al. 1978)

Histopathologic reexamination of livers did not show a carcinogenic effect from exposure to TCI. Hepatocellular carcinomas were found in 3/99 control male and 3/99 low-dose male rats. Histopathologic reexamination of other tissues was not evident.

8.1.4 Inhalation Exposure: Hamsters

8.1.4.1 Henschler et al. (1980)--Henschler et al. (1980) conducted a carcinogenicity study on TCI in Syrian hamsters. The TCI sample and the experimental conditions were those used in the carcinogenicity study of TCI in Han:Wist rats by Henschler et al. (1980) discussed herein. Thirty male and 30 female hamsters per exposure group were exposed to 0 (controls exposed to inhalation chamber air only), 100, or 500 ppm TCI vapor 6 hours daily, 5 days per week, for 18 months. The initial age of the animals was not given. Terminal sacrifice was at 30 months. All animals were subjected to necropsy and histopathologic examination.

No effect of TCI on body weight gain was observed. A treatment-related effect on survival of hamsters was not found (Table 8-16); however, a marked increase in the death rate for all groups ensued after 75 weeks.

A carcinogenic effect of TCI was not evident in this study. Use of a maximum tolerated dose is not indicated in the study report, and the similarity of survival and body weight between control and treatment groups indicates that higher exposure levels of TCI possibly could have been used to provide a broader evaluation of TCI carcinogenicity.

8.1.5 Inhalation Exposure: Mice

8.1.5.1 Henschler et al. (1980)--Henschler et al. (1980) conducted a carcinogenicity study on TCI in Han:NMRI mice. The TCI sample and the experimental conditions were those used in the carcinogenicity study of TCI in Han:Wist rats by Henschler et al. (1980) discussed herein. Thirty male and 30 female mice per exposure group were exposed to 0 (controls exposed to inhalation chamber air only), 100, or 500 ppm TCI vapor 6 hours daily, 5 days per week, for 18 months. The initial age of the animals was not given. Terminal sacrifice was at 30 months. All animals were subjected to necropsy and histopathologic examination.

TABLE 8-16. ESTIMATION OF PROBABILITY OF SURVIVAL OF HAMSTERS (%)[a]
(Henschler et al. 1980)

	Group					
	Control		100 ppm		500 ppm	
Week	M	F	M	F	M	F
1	100.0	100.0	100.0	100.0	100.0	100.0
50	100.0	100.0	100.0	100.0	100.0	90.0
75	100.0	83.6	100.0	89.7	100.0	76.6
100	56.7	23.3	63.3	20.7	70.0	30.0
115	40.0	6.7	26.7	-	40.0	-
126	13.3	-	6.7	-	26.7	-
156	-	-	-	-	-	-

[a] By the Kaplan and Meier (1958) method.

No effect on body weight gain was observed. Survival patterns described in Table 8-17 show a greater mortality in treated mice compared to controls, particularly after 50 weeks in males and 75 weeks in females. A statistically significant ($P < 0.05$) decrease in survival rates of males and females in both treatment groups compared to controls was reported. The survival patterns indicate that the TCI exposure levels used were toxic to male mice; increased mortality in treated females was particularly evident during the period when lymphoma incidence was increasing in these groups.

TABLE 8-17. ESTIMATION OF PROBABILITY OF SURVIVAL OF MICE (%)[a]
(Henschler et al. 1980)

Week	Group					
	Control		100 ppm		500 ppm	
	M	F	M	F	M	F
1	100.0	100.0	100.0	100.0	100.0	100.0
50	93.3	93.3	86.7	96.6	83.3	100.0
75	83.3	83.3	63.3	73.3	56.6	82.8
100	66.7	46.7	36.6	30.0	26.7	37.9
115	33.3	33.3	16.6	13.3	13.3	10.3
126	23.3	26.7	10.0	3.3	3.3	3.4
156	-	-	-	-	-	-

[a] By the Kaplan and Meier (1958) method.

A carcinogenic effect of TCI was not evident in male mice in this study; however, the incidence (number affected/number examined) of malignant lymphomas in female mice was as follows: 9/29 (control), 17/30 (100 ppm), and 18/28 (500 ppm). The response in the low ($P = 0.042$) and high ($P = 0.012$) dose groups represents a statistically significant ($P < 0.05$) increase over the control incidence by the Fisher Exact Test. The time-to-tumor occurrence was shorter in treated females; however, the majority of lymphomas were apparent after treatment was terminated at 78 weeks, as illustrated in Figure 8-12. Induction of hepatocellular carcinomas by exposure to TCI was not observed in this study. Hepatocellular adenoma was diagnosed in one control and two low-dose male mice, and hepatocellular carcinoma was discovered in one control male mouse.

According to the authors, the increased incidence of malignant lymphoma in female mice could have been due to an occurrence of immunosuppression capable

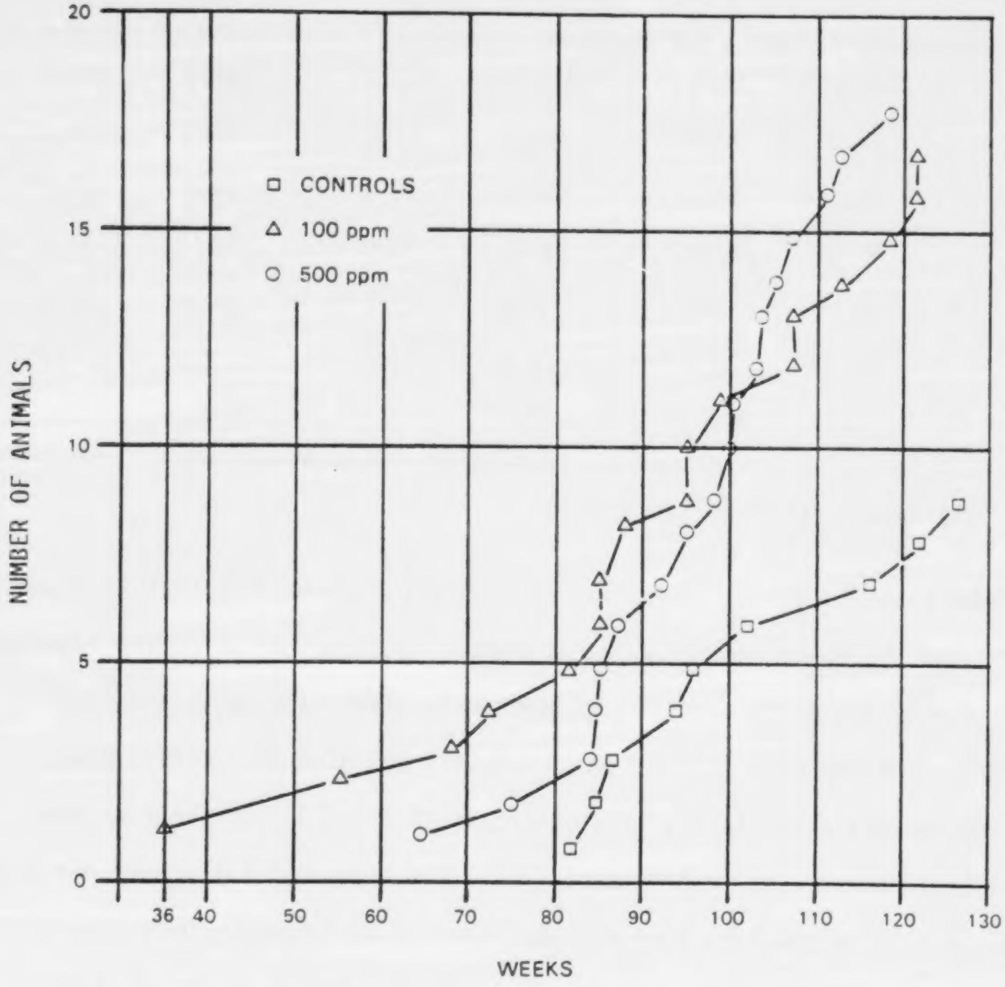

Figure 8-12. Incidence of malignant lymphomas in female mice.

of enhancing the susceptibility of tumor induction by specific, mostly inborn, viruses. The 30% incidence of malignant lymphoma in matched control female mice in the Henschler et al. (1980) study is higher than the 12 to 22% (average 16%) range of lymphoma incidence determined by Luz (1977) between 1968 and 1977 for 830 untreated female NMRI-Neuherberg mice used as control groups in long-term experiments. It was the authors' impression that it was not clear whether an immunosuppressive effect would have been exerted by TCI itself or by other nonspecific influences, such as stress.

Under the conditions described in the report of their study, Sanders et al. (1982) indicated an ability of orally administered TCI to reduce the immune status in CD-1 mice; therefore, enhancement of the lymphoma incidence in the treated female mice possibly could have been related to an immunosuppressive effect from TCI treatment. Kreuger (1972) presented an argument similar to that of Henschler et al. (1980) in which a coexistence of persistent antigenic stimulation and chronic immunosuppression is pathogenetically related to malignancy in immunocompetent tissues. Furthermore, Kreuger (1972) indicated that a significant increase in the incidence of lymphoreticular neoplasms corresponded to a significantly shortened latent period of tumor formation in several experiments cited in his report. The argument of Henschler et al. (1980), therefore, provides a possible interpretation of the results of their study.

8.1.5.2 Bell et al. (1978; audit of Industrial Bio-Test Laboratories, Inc. study)--A carcinogenicity study of TCI vapor in B6C3F1 mice was conducted at Industrial Bio-Test Laboratories, Inc. (IBT) from 1975 to 1977, and audit findings by the Manufacturing Chemists Association (MCA) have been reported (Bell et al. 1978). The TCI test sample and the experimental design were those used in the IBT study in Charles River rats discussed elsewhere herein. Each of three treatment groups (exposed to nominal levels of 100, 300, or 600 ppm TCI vapor, 6 hours daily, 5 days/week, for 24 months), as well as an untreated control group, consisted of 140 mice of each sex. Except for 40 mice/sex/group assigned to interim sacrifice for evaluation of clinical chemistry and hematology, a pathologic examination was scheduled for each animal. Surviving animals were sacrificed on termination of treatment.

Serious flaws in the conduct of this study were described in the audit report by the MCA. Wide deviations in actual exposure levels compared to nominal concentrations and high variability in the number of vapor measurements made daily, as found in laboratory records made early in the study, are described in the discussion of the IBT carcinogenicity study in rats. Furthermore, although all mice evidently were received from the same supplier, control mice appear to have been selected from a shipment obtained approximately 3 weeks earlier. Hence, the control group was not specifically matched with the treatment groups. Histopathologic reexamination of livers revealed 39 discrepancies between gross and microscopic observations, which were mostly the result of improper sectioning of liver containing tumors found grossly. Twelve mice ($\leq$ 3 per group) were identified as originally missexed during reexamination of liver specimens. The sex of nine mice ($\leq$ 2 per group) could not be determined, and for the tabulation of liver tumor data, the sex shown on laboratory records was assumed.

Statistical analysis of liver tumor data from reexamination of liver pathology was done by the Fisher Exact Test and the chi-square analysis (Table 8-18). Significant ($P < 0.05$) increases in the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma/hepatocellular carcinoma combined in treated males compared to untreated males and the incidence of hepatocellular adenoma/hepatocellular carcinoma combined in treated females

TABLE 8-18. STATISTICAL ANALYSIS OF THE INCIDENCE OF PRIMARY HEPATOCELLULAR NEOPLASMS IN CONTROL AND TRICHLOROETHYLENE TREATED B6C3F1 MICE (Bell et al. 1978)

	Untreated controls	T-1[a]	T-2[b]	T-3[c]	T-1 vs. T-2	T-1 vs. T-3	T-2 vs. T-3
			Males				
Hepatocellular adenoma							
Incidence	2/99	7/95	7/100	10/97			
Percent	2.02%	7.37%	7.00%	10.31%			
Chi square		2.042	1.820	4.504*	0.032	0.214	0.329
Fisher Exact Test		0.0752	0.0873	0.0150*	0.6465	0.3223	0.2834
Hepatocellular carcinoma							
Incidence	18/99	28/95	31/100	43/97			
Percent	18.18%	29.47%	31.00%	44.33%			
Chi square		2.821	3.741	14.431*	0.006	3.930*	3.184
Fisher Exact Test		0.0463*	0.0262*	0.000063*	0.4698	0.0235*	0.0371*
Hepatocellular adenoma/ hepatocellular carcinoma combined							
Incidence	20/99	35/95	38/100	53/97			
Percent	20.20%	36.84%	38.00%	54.64%			
Chi square		5.815*	6.794*	23.408*	0.000	5.427*	4.836*
Fisher Exact Test		0.00778*	0.004407*	0.00000049*	0.4933	0.0098*	0.0138*
			Females				
	Untreated controls	T-1	T-2	T-3	T-1 vs. T-2	T-1 vs. T-3	T-2 vs. T-3
Hepatocellular adenoma							
Incidence	2/99	5/100	1/94	4/99			
Percent	2.02%	5.00%	1.06%	4.04%			
Chi square		0.572	0.002	0.172	1.364	0.000	0.719
Fisher Exact Test		0.2265	0.8672	0.3413	0.9824	0.7461	0.2007
Hepatocellular carcinoma							
Incidence	6/99	4/100	9/94	13/99			
Percent	6.06%	4.00%	9.57%	13.13%			
Chi square		0.116	0.1418	2.096	1.599	4.205*	0.303
Fisher Exact Test		0.8382	0.2604	0.0730	0.1026	0.0183*	0.2916
Hepatocellular adenoma/ hepatocellular carcinoma combined							
Incidence	8/99	9/100	10/94	17/99			
Percent	8.08%	9.00%	10.64%	17.17%			
Chi square		0.000	0.132	2.930	0.020	2.250	1.211
Fisher Exact Test		0.5083	0.3579	0.0427*	0.4427	0.0662	0.1353

*Significant at 95% level.
[a] T-1: 100 ppm
[b] T-2: 300 ppm
[c] T-3: 600 ppm

compared to untreated females were calculated as shown in Table 8-18. With respect to the treatment groups which were on study concurrently, statistically significant ($P < 0.05$) increases in both the number of hepatocellular carcinomas alone and hepatocellular adenomas/hepatocellular carcinomas combined in high-dose vs. either low- or mid-dose males and in hepatocellular carcinomas in high-dose vs. low-dose females were found. Increases in liver tumor incidence in high-dose compared to low-dose mice may be related to clustering of exposure level frequencies around nominal concentrations, as shown in Figures 8-9, 8-10, and 8-11 in the discussion of the IBT rat study, and increased hepatocellular carcinoma incidence was evident in B6C3F1 mice treated with TCI by gavage in the NTP (1982) and NCI (1976) bioassays discussed in this document. However, this study is weakened by the deficiencies in its conduct described above. An ongoing carcinogenicity study by Dr. C. Maltoni and associates can provide further evaluation of the carcinogenic potential of TCI vapor in BC63F1 mice.

8.1.6 Other Carcinogenicity Evaluations of Trichloroethylene--Although done mainly to estimate general toxic effects of TCI, as discussed elsewhere in this document, the studies summarized in Table 8-19, except for that by Laib et al. (1978), were described by Waters et al. (1977) as studies in which carcinogenic activity for TCI was not demonstrated. However, these studies involved small groups of animals and durations of treatment and observation which were short with respect to the lifetime of the animals or, in the study by Rudali (1967), a duration which was unspecified.

Laib et al. (1979, 1978) histochemically evaluated livers from Wistar rats exposed to either vinyl chloride (VC) or TCI, by the protocol shown in Table 8-19, for focal deficiencies of nucleoside triphosphatase (ATPase) as evidence for pre-malignant hepatocellular lesions. The authors stated that Friedrich-Freksa

TABLE 8-19. SUMMARY OF NEGATIVE CARCINOGENICITY DATA FOR TRICHLOROETHYLENE
(Waters et al. 1977)

Species	Number	Exposure[a]	Results	Reference
Dogs	16	Inhalation 150-750 ppm in air 20-48 hr/wk for 7-16 wk	No tumors; no deaths	Seifter 1944
Rats Guinea pigs Monkeys Rabbits	12 11 2 4	Inhalation 3,000 ppm, 27 exposures, 36 days 100 ppm, 132 exposures, 85 days 200 ppm, 148 exposures, 212 days 200 ppm, 178 exposures, 248 days	Three rats died; no tumors	Adams et al. 1951
Cats	8	Inhalation 200 ppm, 178 exposures, 248 days	No tumors; no deaths	Mosinger and Fiorentini 1955
Mice	28	Intragastric 0.1 ml in 40% oil solution 2/wk, unspecified treatment period	No tumors; no deaths	Rudali 1967
Rats Guinea pigs Monkeys Rabbits Dogs	15 15 3 3 2	Inhalation 3,825 mg/m^3, 8 hr/day, 5 days/wk, 6 months 189 mg/m^3 continuous for 90 days	No tumors; no deaths	Prendergast et al. 1967
Rats	37 newborn and 2 adults per group	Inhalation 2,000 ppm, 8 hr/day, 5 days/wk, 10 weeks followed by 2 weeks without treatment	No tumorigenic potential	Laib et al. 1979, and Laib et al. 1978[b]

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[a] Surviving animals were sacrificed on approximately the final day of exposure or, in the studies by Laib et al., observation; however, the observation period is not specified in the report by Rudali (1967).

[b] Addition to summary by Waters et al. (1977).

had shown that focal deficiencies of distinct hepatocellular enzymes precede formation of hepatocellular malignancies. There was no effect in adult female rats exposed to either chemical, and ATPase-deficient foci were found in livers from newborn rats exposed to VC, but not in livers from newborn rats exposed to TCI. The authors concluded that this experiment did not indicate an oncogenic potency for TCI in hepatocytes of newborn rats. However, it is not certain whether a longer duration of exposure of newborn rats to TCI could ultimately have resulted in enzyme-deficient foci in liver.

8.1.7 Trichloroethylene Oxide

8.1.7.1 Repeated Skin Application and Subcutaneous Administration: Mice

8.1.7.1.1 Van Duuren et al. (1983). Van Duuren et al. (1983) evaluated the carcinogenicity of six epoxides of structurally related chloroalkenes by repeated skin application and subcutaneous injection in chronic studies with female ICR/Ha mice. The epoxides were *cis*-1-chloropropene oxide (*cis*-CPO); *trans*-1-chloropropene oxide (*trans*-CPO); *cis*-1,3-dichloropropene oxide (*cis*-DCPO); *trans*-1,3-dichloropropene oxide (*trans*-DCPO), trichloroethylene oxide (TCIO); and tetrachloroethylene oxide (PCEO). The epoxides were prepared by a modification of the method of Kline et al. (1978) to obtain pure samples. However, *cis*-DCPO and *trans*-DCPO contained 10 to 15% *m*-dichlorobenzene during the initial 14 months of the study, and TCIO contained 10% dichloroacetyl chloride early in the study. Pure chemicals were stored at -70°C.

Mice were 6 to 8 weeks old when the study began. Six animals were housed per cage. There were 30 mice in each experimental group, except for 100 mice in each untreated control group. Maximum tolerated doses were estimated for the chronic studies from doses not showing clinically apparent toxicity, including histopathologic examination of treatment sites and liver, in preliminary toxicity tests of 6 to 8 weeks duration.

For the chronic skin application study, test sites were shaved initially and about every 2 weeks thereafter. The epoxides were dissolved in 0.1 ml acetone for application to the dorsal skin 3 times each week for the duration of the study at the doses described in Table 8-20, except for PCEO, which, due to its instability in acetone, was applied undiluted to skin followed immediately by application of acetone. In the subcutaneous injection study, epoxides in 0.05 ml pure tricaprylin were administered at the doses shown in Table 8-21 into the left flank once weekly for the duration of the study.

Animals were observed daily and weighed bimonthly. Papillomas were characterized as skin lesions of about 1 mm in diameter which persisted more than 30 days. Each animal was necropsied, and each lesion and tumor was histopathologically examined. Routine sections examined microscopically included skin, liver, stomach, and kidney in the skin application study, and injection site tissue and liver in the subcutaneous injection study.

As shown in Tables 8-20 and 8-21, vehicle and untreated control groups were concurrently on study. Positive control groups were treated with dl-diepoxybutane. The above-mentioned impurities m-dichlorobenzene and dichloroacetylchloride were tested at the levels in which they were present in the epoxides.

Results described in Table 8-20 show no induction of skin tumors from repeated application with TCIO, and no significant ($P < 0.05$) increase in local tumors resulted from subcutaneous injection of TCIO compared to controls (Table 8-21). The positive control animals responded with development of highly significant ($P < 0.0005$, chi-square test) tumor increases in both studies (Tables 8-20 and 8-21). The investigators stated that no significant ($P < 0.05$) incidences of malignant distant tumors were observed.

As discussed by Van Duuren et al. (1983), chloroepoxides have been considered as activated carcinogenic metabolites of chloroalkenes. The negative

TABLE 8-20. CHRONIC SKIN APPLICATION IN MICE
(Van Duuren et al. 1983)

Thirty female ICR/Ha Swiss mice per group were painted on the dorsal skin 3 times weekly with halo-epoxides at the doses indicated in 0.1 ml acetone by micropipet except where noted.

Compound	Time (days) to first tumor	Median survival time/test duration in days	No. of mice with papilloma/total papillomas	No. of mice with squamous cell carcinoma	P value[a]
cis-CPO, 10 mg	225	>432/432	14/20[b]	10	<0.0005
trans-CPO, 10 mg	345	513/574	15/20	10[c]	<0.0005
cis-DCPO, 10 mg	371	522/574	16/19	10	<0.0005
trans-DCPO, 10 mg	255	511/573	20/27[d]	17[e]	<0.0005
TCIO, 2.5 mg		526/575	0		
PCEO, 7.5 mg[f]	268	>459/459	3/39	1	0.014
m-Dichlorobenzene, 3.5 mg		520/575	0		
Dichloroacetyl chloride 250 ug		>576/576	0		
dl-Diepoxybutane, 3.3 mg (positive control)	147	462/573	23/63	21[h]	<0.0005
Acetone, 0.1 ml		>576/576	0		
No treatment (100 animals)		551/580	0		

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[a] P values based on number of animals with benign and malignant tumors: X^2 test with Yates' correction was used.

[b] Three were keratoacanthomas.

[c] One animal developed a fibrosarcoma and a squamous cell carcinoma.

[d] One was a histiocytoma, and one was a keratoacanthoma.

[e] One was a fibrosarcoma in the treatment area.

[f] PCEO was applied at 5 ul (7.5 mg) by Eppendorf pipet followed immediately by 0.1 ml acetone by micropipet.

[g] One was a keratoacanthoma.

[h] Two were fibrosarcomas in the treatment area.

TABLE 8-21. CHRONIC S.C. INJECTION IN MICE
(Van Duuren et al. 1983)

Thirty female ICR/Ha Swiss mice per group were given injections once weekly of halo-epoxides s.c. in the left flank at the doses indicated in 0.05 ml tricaprylin

Compound	Median survival time/test duration in days	No. of animals with local tumors	P value[a]
cis-CPO, 1 mg	482/551	1 fibrosarcoma 1 carcinoma[b] 1 leukemia	0.052
trans-CPO, 1 mg	418/556	4 fibrosarcomas 1 carcinoma	0.003
cis-DCPO, 500 ug	500/557	3 fibrosarcomas 1 anaplastic sarcoma 1 carcinoma and mammary tumor	0.003
trans-DCPO, 500 ug	498/557	5 fibrosarcomas[c]	0.003
TCIO, 500 ug	547/558	1 fibrosarcoma	NS[d]
PCEO, 500 ug	491/558	1 anaplastic sarcoma 1 leukemia	NS
m-Dichlorobenzene, 165 ug	525/560	0	
Dichloroacetyl chloride, 50 ug	>560/560	1 fibrosarcoma	NS
dl-Diepoxybutane, 330 ug (positive control)	475/553	9 fibrosarcomas[e] 2 carcinomas 1 mammary tumor	<0.0005
Tricaprylin, 0.05 ml	>524/561	0	
No treatment (100 animals)	551/580	1 fibrosarcoma	

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[a] All mice with local tumors included in calculation of P values: X^2 test with Yates' correction was used. Each treated group was compared with the untreated control group. C-CPO was marginally significant using the 0.050 level as cutoff point.
[b] Squamous cell carcinomas.
[c] One with a focus of angiosarcoma.
[d] NS = not significant, and P > 0.20.
[e] One with a focus of anaplastic sarcoma.

results for carcinogenicity with TCIO in the study by Van Duuren et al. (1983) reflect the negative results for carcinogenicity with TCI reported by Van Duuren et al. (1978) using the same experimental design. Presentation of data for each epoxide tested by Van Duuren et al. (1983) is made herein to provide evidence that the relative carcinogenic activity of the six epoxides was not specifically dependent on stereochemistry (*cis* or *trans*) or rate of hydrolysis (see Figure 8-14 herein). The authors further noted that vinyl chloride epoxide has a half-life in aqueous solution similar to that of TCIO, but that the former compound has demonstrated carcinogenicity by subcutaneous injection and initiating activity in an initiationpromotion study. As indicated by the authors, the epoxides tested would be direct-acting agents which would not have to rely on metabolic activation at the sites of carcinogenic action in their study. Under the conditions of the study by Van Duuren et al. (1983), TCIO was not carcinogenic in female ICR/Ha mice. Routine histopathologic examination of additional tissues and organs might have given a broader evaluation of pathology.

8.1.8 Cell Transformation Studies

8.1.8.1 Trichloroethylene

8.1.8.1.1 Price et al. (1978). Price et al. (1978) evaluated the potential of TCI to transform Fischer rat embryo cells (F 1706, subculture 108) *in vitro*. The TCI sample, obtained from Fisher Scientific Co., was $\geq$ 99% pure and would have been stabilized with 20 ppm triethylamine or 25 ppm diisopropylamine (personal communication with Fisher Scientific Co.). Cells were grown in Eagle's minimum essential medium in Earle's salts supplemented with 10% fetal bovine serum, 2mM L-glutamine, 0.1 mM nonessential amino acids, 100 U penicillin, and 100 ug streptomycin per ml. Liquid TCI was diluted 1:1000 in growth medium before exposure of cells to desired TCI doses.

Doses for the transformation assays were selected as those producing minimal reduction in plating efficiency relative to medium-only control cells in preliminary tests. In the preliminary tests, triplicate cultures were exposed to 10-fold dilutions of TCI for 4 days before fixing and staining of cells and counting of colonies. Minimal reduction in plating efficiency (3 to 16%) compared to controls was produced over the range of TCI doses tested; hence, the two highest doses (1.1×10^4 and 1.1×10^3 uM) of TCI were selected for the transformation assay.

In the transformation assay, quadruplicate cultures of F1706 cells were treated at 50% confluency with the two selected concentrations of TCI. Cells were exposed to TCI for 48 hours and then washed and refed with growth medium alone. Each culture was considered a separate series. Additional cultures were exposed to the positive control agent 3-methylcholanthrene diluted in acetone (1:1000) and growth medium to a dose of 0.37 uM. Negative control cultures were grown in the presence of acetone (1:1000) in growth medium and in growth medium alone. At each subculture, one set of flasks was retained for 2 weeks without subdivision, and the other set of flasks was subdivided 1:2 each week to yield two new sets of cultures, one for the holding series without subdivision, and one for subdivision. Cell transformation was characterized by progressively growing foci composed of cells lacking contact inhibition and orientation, and by the ability of foci to grow in semi-solid agar four subcultures after treatment. The ability of transformed cells to induce local fibrosarcomas after subcutaneous injection of 1×10^6 cells into newborn Fischer 344 rats was also evaluated.

Transformation of cells exposed to TCI was observed, as well as the ability of transformed cells to grow in semi-solid agar and to induce local fibrosarcomas after subcutaneous injection into rats (Table 8-22). Transformation of cells exposed to the positive control agent 3-methylcholanthrene was

TABLE 8-22. *IN VITRO* TRANSFORMATION OF FISCHER RAT EMBRYO CELL CULTURES BY TRICHLOROETHYLENE
(adapted from Price et al. 1978)

Compound	Dose (uM)	Morphological transformation[a]	Growth in agar at P + 4[b]	Tumor incidence[c] No. of animals with tumors/ No. of animals inoculated
Trichloroethylene	1.1×10^4	+5 (44)	9	13/13 (48)
	1.1×10^3	+5 (44)	8	12/12 (55)
3-Methylcholanthrene	3.7×10^{-2}	+4 (37)	124	12/12 (27)
Acetone	1:1,000	(NA)	0	0/13 (82)
Medium		(NA)	0	ND

[a] Numbers represent subcultures after treatment when transformed foci were first observed. The acetone and medium control cultures were still negative at the termination of the experiment nine subcultures after treatment. Cultures were held for 2 weeks at each subculture and stained prior to screening. Numbers in parentheses represent number of days *in vitro* prior to observation of morphological transformation. NA = not applicable, as there was no observed transformation by subculture 9 (81 days).

[b] Numbers represent the average number of microscopic foci per three dishes. Each dish was inoculated with 50,000 cells and held for 4 weeks at 37°C in a humidified CO_2 incubator prior to staining with 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-2H-tetrazolium chloride.

[c] Animals inoculated subcutaneously with cells that had been treated with chemical five subcultures earlier. Numbers in parentheses represent days postinoculation to 100% tumor incidence. ND = not done.

evident, as shown in Table 8-22. Spontaneous cell transformation was not found in negative control cells (Table 8-22).

Although transformation of F1706 cells in the presence of TCI was observed by Price et al. (1978), the potency of TCI was low relative to that of the positive control chemical, 3-methylcholanthrene. The response to TCI may reflect an ability of this cell line to convert TCI to active metabolites, and further evaluation of the effect of TCI through active metabolites possibly could have been made by additional testing with an exogenous metabolic activation system and by testing the ability of the F1706 cells to produce TCI metabolites. The authors stated that the F1706 cell line, at the subculture used (108), did not require addition of murine type "C" virus and was free of any known infectious virus; however, these cells did contain genetic information of the Rauscher leukemia virus. It is not clear whether cell transformation was induced through action of the test chemicals on the host cells, their integrated oncornavirus genome, or both. Additionally, cells were exposed to liquid TCI diluted in growth medium; therefore, since TCI is a volatile substance, exposure of cells to TCI as a vapor could have indicated how different exposure conditions possibly could have influenced its ability to transform F1706 cells.

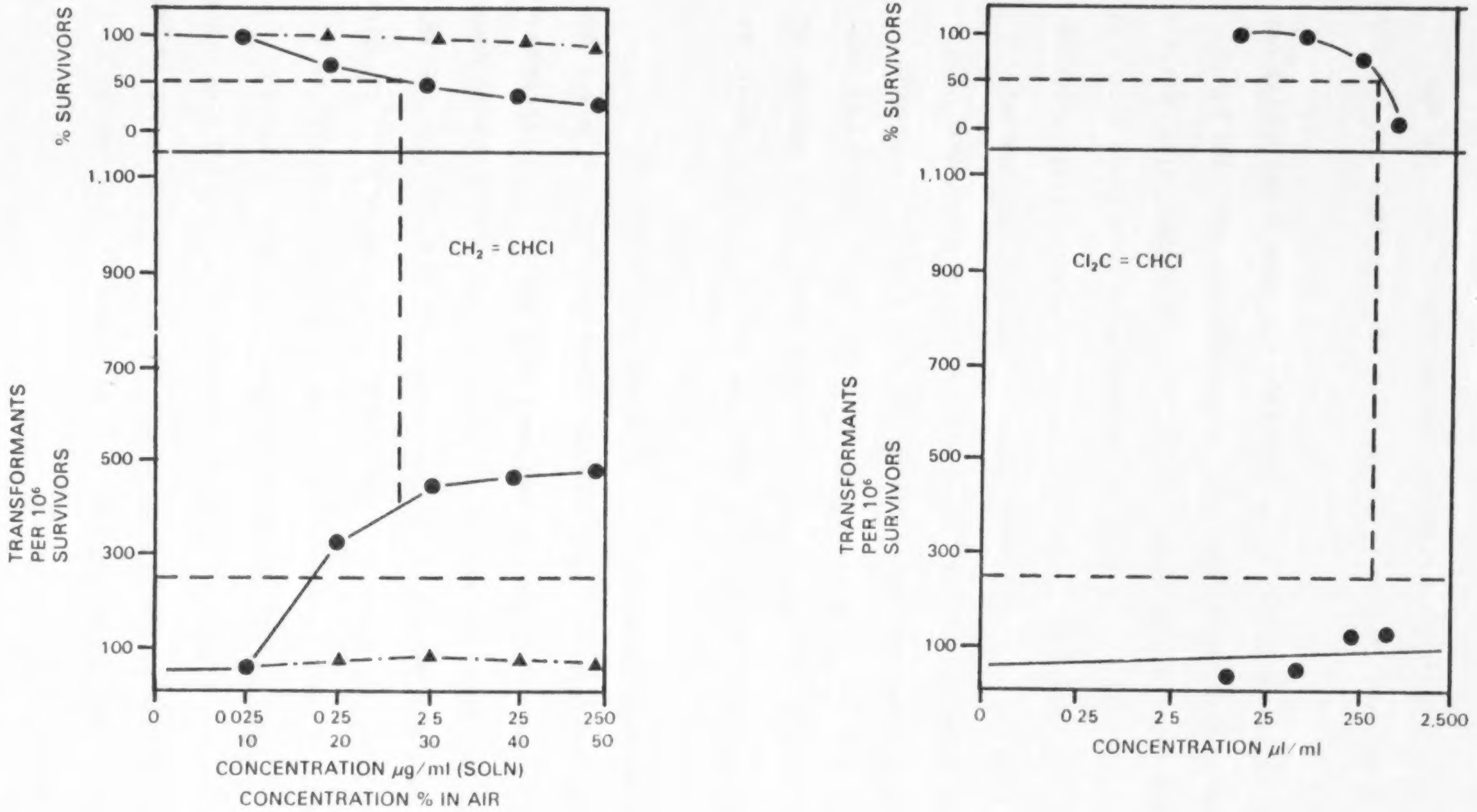
8.1.8.1.2 Styles (1979). Styles (1979) reported an investigation on TCI in a cell transformation system with BHK cells, using growth in semisolid agar as an endpoint, as part of a larger study (Purchase et al. 1978) done to screen chemicals for carcinogenic potential. The BHK-agar transformation assay technique used has been described by Styles (1977) and Purchase et al. (1978). In the study reported by Styles (1979), baby Syrian hamster kidney (BHK-21lC1 13) cells were exposed to five different doses of test substance *in vitro* in serum-free liquid tissue culture medium in the presence of rat liver microsomal fraction and cofactors (S-9 mix; Ames et al. 1975). The liver microsomal fraction was obtained from Sprague-Dawley rats induced with Arochlor 1254.

Cells were grown and maintained in Dulbecco's modification of Eagle's medium in an atmosphere of 20% CO_2 in air. Cells were maintained at 37°C until confluent; subsequently, the cells were trypsinized and resuspended in fresh growth medium. Resuspended cells were grown until 90% confluent for transformation assays or 100% confluent for stock. Only cells with normal morphology were used for assays. To minimize spontaneous transformation frequency, cells were obtained at low passage, grown to 90% confluency, and frozen in liquid nitrogen. Cells were thawed at 37°C in growth medium for further use.

Test compounds were dissolved in DMSO or water as appropriate. Each dose was tested in replicate assays. Cells incubated until 90% confluency were trypsinized and resuspended in Medium 199 at a concentration of 10^6 cells/ml. Resuspended cells (10^6) were incubated with test chemical and S-9 mix at 37°C for 4 hours. To evaluate compound vapors, cells (2.5×10^5) were plated and incubated until 90% confluent, at which time growth medium was replaced with medium 199 and S-9 mix. Duplicate plates were exposed to test chemical vapor at 37°C for 3 hours. After treatment, cells were centrifuged and resuspended in growth medium containing 0.3% agar. Survival after treatment was estimated by incubating 1,000 cells at 37°C for 6 to 8 days before counting colonies. Transformation was evaluated by counting colonies after cells were plated and incubated for 21 days at 37°C. The dose-response for transformation was compared to that for survival. Styles (1977) accepted a fivefold increase in transformation frequency above control values at the LC_{50} as a positive result. The spontaneous transformation frequency of BHK cells (72 experiments) in this study was 50 ± 16 per 10^6 survivors. Suitability of the soft agar medium for colony growth was checked by assays with polyomatransformed BHK-21/Cl 13 cells or Hela cells.

Data reported by Styles (1979) on unstabilized TCI (source and purity not reported) are compared with those found with vinyl chloride (VC) (ICI Mond Division; purity not reported) by Styles (1977, 1979) in Figure 8-13. Results were negative with exposure to TCI solution in DMSO added to culture medium in a dose range including levels in which toxicity was observed. Vinyl chloride was effective as a gas in producing transformation and toxicity; however, VC solution in DMSO added to culture medium was ineffective to suggest that cells were exposed to doses too low to produce an effect by this approach. Although TCI doses high enough to produce toxicity did not induce transformation, exposure of cells to TCI as a vapor could have provided a comparison of the transformation potential of TCI as a vapor and TCI in liquid solution, as was done for VC.

The study by Purchase et al. (1978), which was done on 120 chemicals of various classes, showed that the BHK-agar transformation assay system was about 90% accurate in discriminating between compounds with demonstrated carcinogenic or noncarcinogenic activity, and was in approximately 83% agreement with results from assays done by the authors with *S. typhimurium* (TA 1535, TA 1538, TA 98, TA 100). Styles (1979) and Purchase et al. (1978) indicated, without presenting numerical data, that results were obtained in the *Salmonella* assays on VC in liquid solution, VC gas, and TCI in liquid solution that were similar to results found in the transformation assays. Purchase et al. (1978) also observed that metabolically activated agents transformed BHK cells more strongly in the presence of S-9 mix, thus suggesting that BHK cells have limited intrinsic metabolic capability.



Survival and transformation of BHK cells treated with vinyl chloride gas (●——●) and vinyl chloride solution in DMSO (▲— · —▲).

Survival and transformation of BHK cells treated with unstabilized trichloroethylene solution in DMSO.

Figure 8-13. Cell transformation assays on vinyl chloride and trichloroethylene in BHK-21/Cl 13 *in vitro*. (Styles 1977, 1979)

8.1.8.2 Trichloroethylene Oxide

8.1.8.2.1 DiPaolo and Doniger (1982). DiPaolo and Doniger (1982) assessed the capacity of putative epoxide metabolites of six chloroalkenes, including TCI, to induce morphologic transformation in cultured Syrian hamster cells. The tested epoxides were *cis*-1-chloropropene oxide (c-CPO); *trans*-1-chloropropene oxide (t-CPO); *cis*-1,3-dichloropropene oxide (c-DCPO); *trans*-1,3-dichloropropene oxide (t-CPO), trichloroethylene oxide (TCIO); and tetrachloroethylene oxide (PCEO). TCIO and PCEO were prepared by auto-oxygenation of TCI and PCE with removal of chloride by-products by extraction with 0.5N sodium hydroxide. The other epoxides were synthesized by oxidation of the parent compounds in m-chloroperbenzoic acid. Each epoxide was provided pure, as determined by nuclear magnetic resonance analysis, by Drs. Kline and Van Duuren of New York University.

Cells for culture were obtained from Syrian hamster embryos removed from their mothers on the twelfth and thirteenth days of gestation. Primary cultures were derived by seeding 1 x 10^7 cells/dish from trypsinized embryos. Secondary cultures were made 2 to 3 days later by seeding 5 x 10^6 cells/10 ml complete medium/plate. Mass cultures were grown in modified Dulbecco's minimum essential medium supplemented with 10% fetal bovine serum (FBS) under an atmosphere of 11% carbon dioxide.

For each plate, 300 cells from secondary cultures were plated in complete medium with 20% FBS along with 6 x 10^4 feeder cells previously irradiated as confluent monolayer cultures. After incubation for 2 days, the medium was removed, the cells were washed with phosphate-buffered saline (PBS), and the desired concentration of epoxide in PBS was added to the cells. After incubation of the cells in the presence of epoxide for 30 minutes at 37°C, the PBS was removed, the cells were washed, and fresh complete medium was added. After further incubation for 5 days, the cells were washed with PBS, fixed, and stained with Giemsa. Cell colonies $\geq$ 2 mm in diameter were counted for cloning efficiency and were examined for morphologic transformation, exhibited as criss-crossing and piled-up cells not seen in controls. Transformation frequency was calculated relative to surviving colonies and initial number of cells plated. Twelve dishes of cells were evaluated per dose.

Results of the cell transformation assay with TCIO show a weak transformation effect, with high concentrations (>1 mM) capable of producing cytotoxicity in the form of a dose-related reduction in cell survival (Table 8-23). The weak effectiveness of TCIO at high concentrations may relate to its short stability half-life of 1.3 minutes in aqueous solution (Figure 8-14). However, chemical stability in aqueous solution alone might not have been directly correlated with the ability of the six epoxides to transform cells in this study. The most stable of the six epoxides in aqueous solution, c-DCPO and t-DCPO (Figure 8-14), were the most potent inducers of cell transformation (Table 8-23), but the trans isomer was a stronger inducer than the cis. Additionally, although PCEO and c-CPO had similar stability half-lives in aqueous solutions (Figure 8-14), c-CPO was the stronger inducer of cell transformation, which, as suggested by the authors, may reflect a more limited ability of PCEO to interact with DNA. Although use of a positive control agent was not indicated for this assay, the authors mentioned that transformed colonies found in this study were similar to those induced by benzo[a]pyrene in this cell line in their laboratory.

8.1.9 Summary of Animal and Cell Transformation Studies

Increased incidences of malignant tumors in animals treated with TCI were found in carcinogenicity studies on male and female B6C3F1 mice with gavage or inhalation treatment, female Han:NMRI mice with inhalation treatment, and male

TABLE 8-23. TRANSFORMATION AND SURVIVAL OF SYRIAN HAMSTER EMBRYO CELLS TREATED WITH DIVERSE CHLOROALKENE OXIDES[a]
(DiPaolo and Doniger 1982)

Compound	Dose, mM	Percent survival	Total transformed colonies	Average transformations/ cells seeded, x 10^3
Acetone		100 (29)[b]	0	
c-CPO	0.11	110	4	1.1
	0.27	94	5	1.4
	0.55	90	6	1.7
	1.1	50	12	3.3
t-CPO	0.22	96	0	-
	0.55	78	2	0.56
	1.1	54	6	1.7
	2.2	12	1	0.26
c-DCPO	0.005	92	1	0.26
	0.01	93	3	0.83
	0.02	81	1	0.26
t-DCPO	0.01	89	8	2.2
	0.025	85	8	2.2
	0.05	44	4	1.1
TCEO	1.1	91	1	0.26
	2.5	86	1	0.26
	5.0	73	4	1.1
PCEO	0.87	98	0	-
	4.3	96	3	0.83
	8.7	100	2	0.56
	17.1	70	5	1.4

[a] Plastic dishes (60 mm) were seeded with 300 cells from a secondary culture with irradiated hamster cells (6 x 10^4) for a feeder, grown for 2 days, and incubated with the test chemical in PBS in the absence of growth medium for 30 min at 37°C. Cells were washed, fed again, and incubated for 7 days after which colonies were stained, counted for survival data, and scored for morphologic transformation. Twelve dishes were used for each treatment.

[b] Number in parenthesis is the absolute cloning efficiency of solvent-treated cultures.

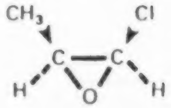
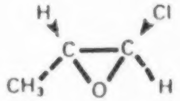
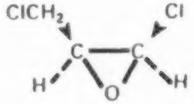
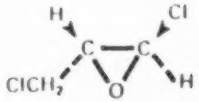
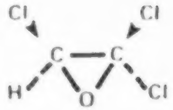
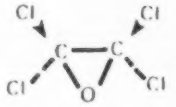
Compound	Structure	Half-Life (min)
cis-1-Chloropropene oxide (*c*-CPO)		11
trans-1-Chloropropene oxide (*t*-CPO)		4.3
cis-1,3-Dichloropropene oxide (*c*-DCPO)		290
trans-1,3-Dichloropropene oxide (*t*-DCPO)		300
Trichloroethylene oxide (TCIO)		1.3
Tetrachloroethylene oxide (PCEO)		12

Figure 8-14. **Structures and stability of chloroalkene oxides. Half-life values are in minutes and were measured at pH 7.4 and 37°C as determined by Kline et al. (1978). (DiPaolo and Doniger 1982)**

Fischer 344 rats with gavage treatment. Incidences of malignant tumors were not observed to be increased in animals treated with TCI in carcinogenicity studies on female Fischer 344 rats, male and female Osborne-Mendel rats, male and female Sprague-Dawley rats, and male and female ICR/Ha Swiss mice, all with gavage treatment; male and female Charles River rats, male and female Han:Wist rats, male and female Syrian hamsters, and male Han:NMRI mice, all with inhalation treatment; female ICR/Ha Swiss mice with subcutaneous injection; and female ICR/Ha Swiss mice with topical application. Increased incidences of malignant tumors were not found in carcinogenicity studies of TCI oxide in female ICR/Ha Swiss mice with subcutaneous injection or topical application. Tumor-initiating activity was not observed in studies where either TCI or TCI oxide was applied to the skin of female ICR/Ha Swiss mice as the initiating agent. Cell transformation activity by TCI in cultured Fischer rat embryo cells and by TCI oxide in Syrian hamster embryo cells was reported. Cell transformation activity for TCI was not found in cultured baby Syrian hamster kidney cells.

8.2 EPIDEMIOLOGIC STUDIES

There are currently nine epidemiologic studies which relate to the issue of TCI exposure and cancer. In only four of these studies, however, were individuals actually identified as having trichloroethylene exposure. Because trichloroethylene has been used to some extent in the dry-cleaning business in the United States, three proportionate mortality studies of decendents who had worked in the dry-cleaning business as well as two case control studies in which the cases and controls were asked about history of employment, including employment in the dry-cleaning business, are also discussed here. In one of the case-control studies, the subjects were also asked about consumption of decaffeinated coffee (TCI has been used as a decaffeinating agent.)

8.2.1 Axelson et al. (1978)

Axelson et al. (1978) found no excess mortality due to cancer in a cohort study of 518 Swedish male TCI production workers. Workers with $\geq$ 10 years of observation were identified as a subcohort. The authors did not indicate the number of workers in this subcohort, but did indicate that the subcohort included 3,643 of the 7,688 total person-years of observation in the study. Since the 1950s the TCI manufacturer had provided, free of charge, two urinary analyses per year per worker of the TCI metabolite trichloroacetic acid (TCA). Additional analyses were reported to have been provided at low cost. Using this information, the subcohort with $\geq$ 10 years of observation was divided into low (average reading of TCA in the urine of less than 100 mg/l) and high (average reading of TCA in the urine of more than 100 mg/l). A level of 100 mg/l of TCA in urine was reported to correspond roughly to an 8-hour time-weighted average exposure of 30 ppm TCI in the air, which is the Swedish occupational standard for TCI. The expected number of deaths was calculated by multiplying the person-years of observation by cause- and age-specific national death rates from the respective calendar years and summing the fractional age and calendar-year contributions over the entire cohort. Table 8-24 compares the expected and observed numbers of deaths for the cohort and subcohort.

There were no significant differences between the observed and the expected numbers of cancer deaths in the total cohort or in any of the subcohorts. The high exposure group, with $\geq$ 10 years of observation, had a risk ratio of 1.7 for cancer deaths, but this was not statistically significant. Two deaths from stomach cancer and two from leukemia occurred in the cohort; the authors did not indicate what the expected number of deaths for these sites would be, however.

Although the data presented in this study do not demonstrate that TCI is a human carcinogen, there are several limitations in the study design, or at least

TABLE 8-24. A COHORT STUDY OF MORTALITY AMONG MEN EXPOSED TO TRICHLOROETHYLENE IN THE 1950s AND 1960s AND FOLLOWED THROUGH DECEMBER 1975[a] (adapted from Axelson et al. 1978)

Cohort	Cause of death	Person-yrs. at observation	Observed	Expected	Risk ratio	Confidence 95% limits risk ratio
Total cohort	All deaths	7688	49	62.0	0.8	0.6 - 1.0
	Tumor deaths		11	14.5	0.8	0.4 - 1.4
Subcohort ($\geq$ 10 years of latency time)	All deaths	3643	37	39.8	0.9	0.7 - 1.3
	Tumor deaths		9	9.5	0.9	0.4 - 1.8
- high exp.	All deaths	548	8	7.6	1.1	0.5 - 2.1
	Tumor deaths		3	1.8	1.7	0.3 - 4.9
- low exp.	All deaths	3095	29	32.2	0.9	0.6 - 1.3
	Tumor deaths		6	7.7	0.8	0.3 - 1.7

[a] Exposure categorization is based on trichloroacetic acid (TCA) in the urine, with low exposure referring to those not exceeding a concentration of 100 mg/l TCA in urine on average.

in the manner in which the data are reported, that would restrict any conclusions regarding this study. For example, little information is available concerning the duration of exposure of the workers. No definition of minimum exposure period seems to have been made by the authors, so that workers who were exposed for a very short period of time could have been included in the study cohort. Secondly, the definition of high and low exposure for the subcohort was based on the average of TCA levels in the urine with no regard to how long the individual may have worked in the high or low exposure area. Thus, an individual may have had very low TCA levels over a short period of time, despite the fact that most of his working time was in a high-exposure area where few samples had been taken. Thirdly, the number of workers in the study (518) was relatively small for the detection of a significant risk of cancer from TCI exposure. Furthermore, it should be noted in this regard that only half of the total person-years of observation were for the group with $\geq$ 10 years of observation, indicating that most of the persons in the cohort had less than 10 years of observation. (It takes more people, when there are fewer person-years per individual, to equal the number of person-years generated by people with more person-years per individual.) Thus, less than 259 people had been followed for as long as 10 years, which itself may not be a long enough observation period in which to observe an increase in cancer mortality, considering that a latency period for cancer may be as long as 20 years. Finally, no analysis was made with regard to specific tumor sites. If TCI is a carcinogen, it is more likely to act on one particular organ or tissue site than on all organ or tissue sites uniformly. It is interesting that 2 of the 11 deaths from cancer in this cohort were from leukemia (18%), whereas approximately 4% of cancer deaths among males in Sweden are leukemia deaths.

In summary, this study does not indicate that TCI is a human carcinogen; however, there are severe limitations in interpreting the data. It should also be noted that the primary author has reported in personal communications (Axelson 1980 and 1983), that the study is being updated through 1980 and that the cohort will be expanded to include 1100 workers who were exposed to TCI between 1970 and 1975.

8.2.2 Tola et al. (1980)

An ongoing cohort study on workers in Finland exposed to TCI was reported by Tola et al. (1980). According to the authors, the cohort is to be followed and analyzed every fifth year. The cohort consisted of 1,148 men and 969 women known to have been occupationally exposed to TCI at some time between 1963 and 1976. Names of workers were obtained from the biochemical laboratory of the Institute of Occupational Health in Finland, where most of the analyses for urinary trichloroacetic acid (TCA), a TCI metabolite, were done as part of the routine medical examinations for workers. Urinalyses were done by the Fujiwara reaction, which was reported to be sensitive to levels as low as 1 mg TCA/l urine. The list of names of persons whose urinary TCA levels had been measured was supplemented with the names of persons who had been reported to have TCI poisoning, according to the Occupational Disease Register of Finland, and with the names of persons who had been reported by employees to have been exposed to TCI. After confirming worker identities, the vital status and the causes of death of those who died as of November 30, 1976 were obtained from the Population Data Register of the Central Statistical Office in Finland, and cancer death information was made available by the Finnish Cancer Registry.

Urinary TCA levels for 2,004 workers were reported. The majority (91%) of the workers had TCA levels below 100 ug/l urine, which, according to Axelson et al. (1978), corresponds to an ambient exposure level of 30 ppm TCI. Furthermore, 78% of the workers had urinary TCA levels below 50 mg/l urine. An additional 80 workers registered with clinical poisoning from exposure to TCI were also part of the cohort; however, urinary TCA measurements were not available for these workers.

The observed numbers of all deaths and of cancer deaths were compared with those expected based on national mortality statistics for 1971. A similar comparison was done for a subcohort of workers exposed before 1970.

Results on the total cohort and subcohort are summarized in Table 8-25. No statistically significant ($P < 0.05$) differences between the observed and the expected numbers of deaths from cancer were found for either the total cohort or the subcohort. Tumor sites for those dying from cancer included the gall bladder, lung, breast, uterus, testis, and multiple myeloma. Two workers with more than 100 ug TCA/l urine and one worker with clinical poisoning had cancer; however, the difference of three cases from the expected number was not statis-

TABLE 8-25. COMPARISON OF WORKERS EXPOSED TO TRICHLOROETHYLENE WITH THE TOTAL FINNISH POPULATION FOR MORTALITY FROM ALL CAUSES AND FOR CANCER MORTALITY (adapted from Tola et al. 1980)

	All deaths		Neoplasms	
Person-years	Expected	Observed	Expected	Observed
Total cohort				
Males				
7,041	52.1	40.0	6.6	5.0
Females				
6,892	32.2	18.0	7.7	6.0
Subcohort (workers exposed before 1970)				
Males				
4,269	36.1	33.0	6.3	5.0
Females				
4,822	25.8	13.0	6.0	4.0

tically significant ($P < 0.05$).

An excess cancer incidence among workers exposed to TCI was not found in this study. The study, however, had a number of limitations. The duration of follow-up was short; the age structure of the cohort was such that 60% of the males and 40% of the females were less than 40 years old at the end of follow-up; the possibility exists, as indicated by the authors, that some workers with low urinary TCA levels actually could have been exposed to another degreasing solvent but were included due to the use of an improper exposure test by the attending physician; and the actual durations of exposure were unknown.

8.2.3 Malek et al. (1979)

Malek et al. (1979) studied the incidence of cancer among a group of 57 dry-cleaners in Prague, Czechoslovakia. This group was reported to represent 86% of all men in Prague who had spent at least one year working in this type of service since the 1950s. The follow-up was from 5 to 50 years with 50% of the workers reported to have been followed for more than 20 years. Six cases of cancer were reported for the group, which was reported to be not significantly ($P < 0.05$) different from what would be expected. The expected number of cancer cases was not included in the authors' report, however. The primary weakness of this study is, of course, the small sample size, with the resulting weakness in the power of the study to detect a significant increase in cancer incidence above that expected.

8.2.4 Paddle (1983)

Paddle (1983) obtained from the Mersey Regional Cancer Registry in England a list of all the liver cancer cases for the period 1951-77. Information on the cases included name, address at registration, and dates of birth and registration. This list was then reduced to those who had both primary liver cancer and an address near Runcorn, which is the location of an Imperial Chemical Industries (ICI) plant that has manufactured trichloroethylene since 1909. This resulting list of 95 was then compared with a list of the "tens of thousands" of employees of the Runcorn plant who had worked during the period 1934-76. None of the 95 primary liver cancer cases who had lived near Runcorn were found to have worked in the ICI plant. As a further check, the files in the company's computerized medical records system were searched for records of past employees dying with liver cancer as the underlying cause. One liver cancer death was found for the Runcorn plant and one liver cancer was found for a neighboring plant. The cancer registry records, however, showed that the first of these had suffered a primary cancer of the esophagus with a secondary liver tumor; the other patient had had multiple secondary deposits from an unknown primary tumor.

There are several deficiencies in this study. In addition, there are limitations in the author's description which make the study difficult to evaluate. One problem is that the author chose only those individuals from the cancer registry who had addresses near Runcorn. Obviously, had a worker lived farther away or moved from Runcorn following employment at the ICI plant, he would not have been included in the study. Another problem is that the author provides no indication as to the likelihood of finding an ICI worker with TCI exposure among the 95 primary liver cancer cases. Although the author reported that the list of 95 liver cancer cases was compared with a list of "tens of thousands" of employees at the ICI plant at Runcorn, he also indicated in his report that only about "1,000 or so" had TCI exposure. No controls were used in the study; thus there is no basis for a statistical comparison. Furthermore, it is not known whether the personnel lists used for comparison included the names of all workers who had worked in the ICI plant during the period 1934-76 or only the names of those who had retired from the plant. If the lists included only the names of retirees, then workers who may have developed liver cancer but left employment before retiring would not have been included in the study. Lastly, for those workers with TCI exposure, it is not known how long they were exposed, or how recent their exposures might have been.

8.2.5 Blair et al. (1979)

Blair et al. (1979) reported, as the preliminary results of a cohort study of 10,000 laundry and dry-cleaning workers, a proportionate mortality analysis of 330 of the workers, who had died during the period 1957-77. Deaths were identified from the mortality records of two union locals in St. Louis, Missouri. The distribution by cause of death among the 330 was compared to that expected based on the proportionate mortality experience of the U.S. Only 279 of these 330 had worked exclusively in dry-cleaning shops; however, the authors did not report the proportionate mortality analyses of these 279. Furthermore, it is unknown what dry-cleaning agent the dry-cleaners in this study may have used. Among the 330 deaths, deaths from cancer of the lung, cervix, and skin contributed to the significant ($P < 0.05$) excess proportion of deaths from cancer at all sites that was found. For lung cancer, there were 17 observed deaths versus 10 expected ($P < 0.05$), for cervical cancer there were 10 observed deaths versus 4.8 expected ($P < 0.05$), and for skin cancer there were 3 observed deaths versus 0.7 expected ($P < 0.05$). On the other hand, a significant deficit of deaths occurred in the cause category identified as "all circulating diseases," with 100 observed versus 125.9 expected ($P < 0.005$), which could account for the excess proportion of cancers.

Conclusions from this study regarding the potential carcinogenicity of TCI are obviously limited. The distribution of deaths for dry-cleaners alone was not reported. Furthermore, it is unknown what dry-cleaning agent these workers may have used. Lastly, confounding variables, such as smoking with regard to the lung cancer excess, socioeconomic status with regard to the cervical cancer excess, and sunlight exposure with regard to the skin cancer excess, were not considered in the analysis of the results.

8.2.6 Katz and Jowett (1981)

Katz and Jowett (1981) analyzed the death certificate records of 671 white female laundry and dry-cleaning workers for the period 1963-1977. Dry-cleaning workers, who are more likely to experience exposure to TCI than laundry workers, could not be separated from laundry workers because they shared the same occupational code. Furthermore, the authors did not state what dry-cleaning agent the dry-cleaning workers used.

Cause-specific proportionate mortality for the deceased laundry and dry-cleaning workers was compared to that for the deaths of all other working females in Wisconsin during the same time period, and to deaths of females in "lower-wage occupations" in Wisconsin during the same time period. Significantly elevated proportionate mortality ratios were found for deaths from cancer of the genitals (unspecified) ($P < 0.01$) and for deaths from cancer of the kidney ($P < 0.05$) when both deaths among women of all occupations and deaths among women of "lower-wage occupations" were used as the comparison groups.

In summary, this study, although suggesting an association of employment in the dry-cleaning industry with an excess risk of certain types of cancer, cannot be said to demonstrate an association between TCI exposure and an excess risk of cancer because of the possible confounding influence of other dry-cleaning agents and because of the inability, from the data available for study, to distinguish between laundry and dry-cleaning workers.

8.2.7 Lin and Kessler (1981)

Lin and Kessler (1981) did a case-control study of 109 pancreatic cancer cases diagnosed during the time period 1972-75 from 115 hospitals in five metropolitan areas. Controls were subjects free of cancer but similar in age (± 3 years), sex, race, and marital status, and were selected at random from among contemporary admissions to the same hospital. Cases and controls were asked about demographic characteristics, residental history, toxic exposures, animal contacts, smoking habits, diet, medical history, medications, and family history. Males were asked about sexual practices and urogenital conditions. Females were questioned on these topics and also on their marital, obstetric, and gynecologic practices. Among other statistically significant associations, an association was found between pancreatic cancer and the drinking of decaffeinated coffee. TCI has been used as a decaffeinating agent, but it is not known whether the coffee that was drunk by the pancreatic cancer cases was decaffeinated with TCI, or if it was, whether the decaffeinated coffee was contaminated with

TCI. An association was also found between pancreatic cancer and employment either as a dry-cleaner or in a job involving exposure to gasoline. It is not known, however, how many pancreatic cancer cases were employed as dry-cleaners and how many were employed in occupations involving exposure to gasoline. Furthermore, the dry-cleaners may have used a variety of cleaning agents. Thus this study, although suggesting an association between employment as a dry-cleaner and an excess risk of pancreatic cancer, cannot be said to demonstrate an association between pancreatic cancer and TCI exposure.

8.2.8 Asal (personal communication 1983)

Two cancer epidemiology studies conducted by Dr. Nabih Asal of the University of Oklahoma found an excess of cancer among dry-cleaners (personal communication 1983). Published reports of the studies are not yet available, however. One of the studies was a kidney cancer case-control study in which Asal found an association between kidney cancer and employment as a dry-cleaner. The second study was a proportionate mortality study of dry-cleaning workers in Oklahoma. Asal reported that results from that study suggested an increased mortality from kidney and lung cancer among the workers. Again, as in the Blair et al., Katz and Jowett, and Lin and Kessler studies, exposure in the studies by Asal was not specific for TCI. Moreover, the conclusions of the study cannot objectively be evaluated until published descriptions of the studies are available.

8.3 QUANTITATIVE ESTIMATION

This quantitative section deals with the unit risk for TCI in air and water and the potency of TCI relative to other carcinogens that the CAG has evaluated. The unit risk estimate for an air or water pollutant is defined as the lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 ug/m^3 of the agent in the air they breathe, or to 1 ug/l in the water they drink. This calculation is done to estimate in quantitative terms the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other, and 2) to give a crude indication of the population risk which might be associated with air or water exposure to these agents, if the actual exposures are known.

These evaluations are made independently of the overall weight of evidence that TCI is carcinogenic in animals. Therefore, the attempt here to do a quantitative estimate should not be interpreted as implying that the evidence for carcinogenicity is strong. For TCI that evidence is limited.

8.3.1 Procedures for Determination of Unit Risk

The data used for quantitative estimation are one or both of two types: 1) lifetime animal studies, and 2) human studies where excess cancer risk has been associated with exposure to the agent. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses with an incidence determined by the extrapolation model.

There is no solid scientific basis for any mathematical extrapolation model that relates carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. For practical reasons such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. We must, therefore, depend on our current understanding of the mechanisms of carcinogenesis for guidance as to which risk model to use. At the present time the dominant view of the carcinogenic process involves the concept that most agents that cause cancer also cause irreversible damage to DNA. This position is reflected by the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal type of biological response, which is characteristic of mutagenesis, is associated with a linear non-threshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at higher doses, there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The linear non-threshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxins in the diet). There is also some evidence from animal experiments that is consistent with the linear non-threshold model (e.g., liver tumors induced in mice by 2-acetylaminofluorene in the large scale ED_{01} study at the National Center for Toxicological Research and the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because its scientific basis, although limited, is the best of any of the current mathematical extrapolation models, the linear non-threshold model has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with this model should be regarded as conservative, representing the most plausible upper limit for the risk, i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation chosen to describe the linear non-threshold dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

8.3.1.1 Animals

8.3.1.1.1 Description of the low-dose animal extrapolation model. Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp [-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp [-(q_1 d + q_2 d^2 + \ldots + q_k d^k)]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d or the effect of treatment.

The point estimate of the coefficents q_i, $i = 0, 1, 2, \ldots, k$, and consequently the extra risk function $P_t(d)$ at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL79, developed by Crump and Watson (1979). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1 \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk and R/q_1^* is a 95% lower confidence limit on the dose producing an extra risk of R. Let L_0 be the maximum value of the log-likelihood function. The upper limit, q_1^*, is calculated by increasing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value, q_1^*, for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2\ (L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper-limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear non-threshold concept discussed earlier. The slope, q_1^*, is taken as an upper-bound of the potency of the chemical in inducing cancer at low doses. (In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P.)

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose is deleted, and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i\ (1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever X^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

8.3.1.1.2 Selection of data. For some chemicals, several studies in different animals species, strains, and sexes, each run at several doses and different routes of exposure, are available. A choice must be made as to which of the data sets from several studies to use in the model. It may also be appropriate to correct for metabolism differences between species and absorption factors via different routes of administration. The procedures used in evaluating these data are consistent with the approach of making a maximum-likely risk estimate. They are listed as follows.

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is statistically significantly higher than the control for at least one test dose level and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, q_1^*, is selected in most cases. However, efforts are made to exclude data sets that produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship, and one has a very small sample size, the set of data that has the larger sample size is selected for calculating the carcinogenic potency.

2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex, and tumor sites, the geometric mean of q_1^*, estimated from each of these data sets, is used for risk assessment. The geometric mean of numbers $A_1, A_2, \ldots, A_m$ is defined as

$$(A_1 \times A_2 \times \ldots \times A_m)^{1/m}$$

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

8.3.1.1.3 Calculation of human equivalent dosages from animal data. Following the suggestion of Mantel and Schneiderman (1975), we assume that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, the surface area is proportional to the 2/3rds power of the weight as would be the case for a perfect sphere, the exposure in mg/day per 2/3rds power of the weight is also considered to be equivalent exposure. In an animal experiment this equivalent dose is computed in the following manner.

Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e), and

W = average weight of the experimental animal

Then, the lifetime average exposure is

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

8.3.1.1.3.1 Oral. Often exposures are not given in units of mg/day and it becomes necessary to convert the given exposures into mg/day. For example, in most feeding studies exposure is in terms of ppm in the diet. Similarly in drinking water studies exposure is in ppm in the water. In these cases the exposure in mg/day is

$$m = ppm \times F \times r$$

where ppm is parts per million of the carcinogenic agent in the diet or water, F is the weight of the food or water consumed per day in kg, and r is the absorption fraction. In the absence of any data to the contrary, r is assumed to be equal to one. For a uniform diet, the weight of the food consumed is proportional to the calories required, which in turn is proportional to the surface area or 2/3rds power of the weight. Water demands are also assumed proportional to the surface area, so that

$$m \propto ppm \times W^{2/3} \times r$$

or

$$\frac{m}{rW^{2/3}} \propto ppm$$

As a result, ppm in the diet or in water is often assumed to be an equivalent exposure between species. However, we feel that this is not justified since the calories/kg of food are very different in the diet of man compared to laboratory animals primarily due to moisture content differences. Consequently, the amount of drinking water required by each species differs also because of the amount of moisture in the food. Therefore, we use an empirically-derived factor, $f = F/W$, which is the fraction of a species body weight that is consumed per day as food or water. We use the following rates:

		Fraction of Body Weight Consumed as	
Species	W	f_{food}	f_{water}
Man	70	0.028	0.029
Rats	0.035	0.05	0.078
Mice	0.03	0.13	0.17

Thus, when the exposure is given as a certain dietary or water concentration in ppm, the exposure in $mg/W^{2/3}$ is

$$\frac{m}{rW^{2/3}} = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}$$

When exposure is given in terms of mg/kg/day = m/Wr = s, the conversion is simply

$$\frac{m}{rW^{2/3}} = s \times W^{1/3}$$

8.3.1.1.4 Calculation of the unit risk from animal studies. The 95% upper-limit risk associated with d $mg/kg^{2/3}$/day is obtained from GLOBAL79 and, for most cases of interest to risk assessment, can be adequately approximated by $P(d) = 1 - \exp-(q_1^*d)$. A "unit risk" in units X is simply the risk corresponding to an exposure of X = 1. To estimate this value we simply find the number of $mg/kg^{2/3}$/day corresponding to one unit of X and substitute this value into the above relationship. Thus, for example, if X is in units of ug/m^3 in the air, we have that for case 1, $d = 0.29 \times 70^{1/3} \times 10^{-3}$ $mg/kg^{2/3}$/day, and for case 2, d = 1, when ug/m^3 is the unit used to compute parameters in animal experiments.

If exposures are given in terms of ppm in air, we may simply use the fact that

$$1 \text{ ppm} = 1.2 \times \frac{\text{molecular weight (gas) mg/m}^3}{\text{molecular weight (air)}}$$

Note, an equivalent method of calculating unit risk would be to use mg/kg/day for the animal exposures and then increase the jth polynomial coefficient by an amount

$$(W_h/W_a)^{j/3} \qquad j = 1, 2, \ldots, k$$

and use mg/kg equivalents for the unit risk values.

8.3.1.1.4.1 Adjustment for less than lifespan duration of experiment. If the duration of experiment, L_e, is less than the natural lifespan of the test animal, L, the slope, q_1^*, or more generally the exponent, g(d), is increased by multiplying a factor $(L/L_e)^3$. We assume that if the average dose, d, is continued, the age-specific rate of cancer will continue to increase as a constant function of the background rate. The age-specific rates for humans increase at least by the 2nd power of the age and often by a considerably higher power as demonstrated by Doll (1971). Thus, we would expect the cumulative tumor rate to increase by at least the 3rd power of age. Using this fact, we assume that the slope, q_1^*, or more generally the exponent, g(d), would also increase by at least the 3rd power of age. As a result, if the slope q_1^* [or g(d)] is calculated at age L_e, we would expect that if the experiment had been continued for the full lifespan, L, at the given average exposure, the slope q_1^* [or g(d)] would have been increased by at least $(L/L_e)^3$.

This adjustment is conceptually consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Crump (1979) where the probability of cancer by age t and at dose d is given by

$$P(d,t) = 1 - \exp\,[-f(t) \times g(d)]$$

8.3.1.1.5 Interpretation of quantitative estimates. For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification regarding carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex, and by the same route of exposure, preferably by inhalation.

The quantitative aspect of the carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, e.g., setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper-limit of risk; i.e., it is not likely that the true risk would be much more than the estimated risk, but it could very well be considerably lower. The risk estimates presented in subsequent sections should not be regarded as an accurate representation of the true cancer risks even when the exposures are accurately defined; however, the estimates presented may be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

8.3.1.1.6 Alternative methodological approaches. The methods used by the Carcinogen Assessment Group (CAG) for quantitative assessment are consistently conservative, i.e., tending toward high estimates of risk. The most important part of the methodology contributing to this conservatism is the linear non-threshold extrapolation model. There are a variety of other extrapolation models that could be used, all of which would give lower risk estimates. These alternative models have not been used by the CAG in the following analysis because it feels that the limited evidence of carcinogenicity for TCI does not warrant that complete an analysis. Furthermore, the CAG feels that with the limited data available from these animal bioassays, especially at the high-dose levels required for testing, almost nothing is known about the true shape of the dose-response curve at low environmental levels. The position is taken by the CAG that the risk estimates obtained by use of the linear non-threshold model are plausible upper limits, and that the true risk could be lower.

In terms of the choice of animal bioassay as the basis for extrapolation, the general approach is to use the most sensitive responder on the assumption that humans are as sensitive as the most sensitive animal species tested. For TCI, the average response of all of the adequately tested bioassay animals was used; this is because three well-conducted valid drinking water studies using different strains of rats showed similar target organs and about the same level of response.

Extrapolations from animals to humans could also be done on the basis of relative weights rather than relative surface areas. The latter approach, used here, has more basis in human pharmacological responses; it is not clear which of the two approaches is more appropriate for carcinogens. In the absence of information on this point, it seems appropriate to use the most generally employed method, which also is more conservative. In the case of the TCI gavage study, the use of extrapolation based on surface area rather than weight increases the unit risk estimates by a factor of 12 to 13.

8.3.1.2 Humans--Model for Estimation of Unit Risk Based on Human Data--If human epidemiologic studies and sufficiently valid exposure information are available for the compound, they are always used in some way. If they show a carcinogenic effect, the data are analyzed to give an estimate of the linear dependence of cancer rates on lifetime average dose, which is equivalent to the facter B_H. If they show no carcinogenic effect when positive animal evidence is available, then it is assumed that a risk does exist, but it is smaller than could have been observed in the epidemiologic study, and an upper limit to the cancer incidence is calculated assuming hypothetically that the true incidence is below the level of detection in the cohort studied, which is determined largely by the cohort size. Whenever possible, human data are used in preference to animal bioassay data.

Very little information exists that can be utilized to extrapolate from high exposure occupational studies to low environmental levels. However, if a number of simplifying assumptions are made, it is possible to construct a crude dose-response model whose parameters can be estimated using vital statistics, epidemiologic studies, and estimates of worker exposures.

In human studies, the response is measured in terms of the relative risk of the exposed cohort of individuals compared to the control group. The mathematical model employed assumes that for low exposures the lifetime probability of death from lung cancer (or any cancer), P_0, may be represented by the linear equation

$$P_0 = A + B_H x$$

where A is the lifetime probability in the absence of the agent, and x is the average lifetime exposure to environmental levels in some units, say ppm. The factor, B_H, is the increased probability of cancer associated with each unit increase of the agent in air.

If we make the assumption that R, the relative risk of lung cancer for exposed workers compared to the general population, is independent of the length or age of exposure but depends only upon the average lifetime exposure, it follows that

$$R = \frac{P}{P_0} = \frac{A + B_H\ (x_1 + x_2)}{A + B_H\ x_1}$$

or

$$RP_0 = A + B_H\ (x_1 + x_2)$$

where x_1 = lifetime average daily exposure to the agent for the general population, x_2 = lifetime average daily exposure to the agent in the occupational setting, and P_0 = lifetime probability of dying of cancer with no or negligible TCI exposure.

Substituting $P_0 = A + B_H \times x_1$ and rearranging gives

$$B_H = P_0\ (R - 1)/x_2$$

To use this model, estimates of R and x_2 must be obtained from the epidemiologic studies. The value P_0 is derived from the age-cause-specific death rates for combined males found in the 1976 U.S. Vital Statistics tables using the life table methodology. For lung cancer the estimate of P_0 is 0.036. This methodology is used in the section on unit risk based on human studies.

8.3.2 Unit Risk from Animal Studies

8.3.2.1 Water--None of the six available epidemiologic studies provided positive results from which to derive a quantitative risk estimate for TCI exposure. For the animal data only the 1982 NTP gavage study and the 1976 NCI gavage study provided sufficient evidence of treatment-related carcinogenicity; both of these studies produced treatment-related hepatocellular carcinomas in male and female B6C3F1 mice. The results of all the carcinogenicity bioassays have been previously presented (Table 8-1). The NTP tumor data for male and female mice have also been presented (Table 8-6 and 8-7). The NCI tumor data for male and female mice have been presented in Table 8-11. These hepatocellular carcinoma data are also shown in Table 8-26, together with the human equivalent dosage

TABLE 8-26. INCIDENCE RATES OF HEPATOCELLULAR CARCINOMAS IN MALE AND FEMALE MICE IN THE NTP (1982) AND NCI (1976) GAVAGE STUDIES. CONTINUOUS HUMAN EQUIVALENT DOSAGES, AND ESTIMATES OF q_1^*, 95% UPPER-LIMIT SLOPE, FROM THE LINEARIZED MULTISTAGE MODEL

	Study	Continuous human equivalent (animal nominal) doses mg/kg/day	Incidence rates No. with tumor/total(%)	95% upper-limit slope estimate q_1^*
Male	NTP	0 (0)	8/48 (17%)	$(mg/kg/day)^{-1}$
		59.3[a] (1,000)	30/50 (60%)	1.8×10^{-2}
	NCI	0 (0)	1/20 (5%)	
		56.3 (1,169)[b]	26/50 (52%)	2.0×10^{-2}[c]
		112.6 (2,339)[b]	31/48 (65%)	
Female	NTP	0 (0)	2/48 (4%)	7.7×10^{-3}
		56.7 (1,000)	13/49 (27%)	
	NCI	0 (0)	0/20 (0%)	
		38.7 (869)[b]	4/50 (8%)	6.5×10^{-3}[c]
		77.3 (1,739)[b]	11/47 (23%)	

[a] All 95% upper-limit slopes q_1^* calculated using continuous human equivalent doses.

$$\text{Equivalent human dosage} = \text{animal dose} \times \frac{5}{7}\text{ days} \times \frac{l_e}{L_e} \times (W_a/70)^{1/3}$$

where W_a = weight of the mice. The average weight of males is taken as 40 grams for dosed males and 35 grams for dosed females for the NTP study; for the NCI study the average weights are 33 grams for males and 26 grams for females. L_e = 2 years and l_e, the duration of exposure, is 2 years for the NTP study and 1.5 years for the NCI study.

[b] Time-weighted average gavage dose over 78-week treatment period; see Table 8-8.

[c] Slope is increased by $(104/90)^3 = 1.54$ due to the duration of the experiment's being less than the natural lifespan of the test animal.

conversions and the 95% upper-limit slope factor, q_1^*. As seen in the table, the largest estimates are those from hepatocellular carcinomas in the males. These values are $q_1^* = 1.8 \times 10^{-2}$ (mg/kg/day)$^{-1}$ from the NTP gavage study and 2.0×10^{-2} (mg/kg/day)$^{-1}$ from the NCI gavage study. Since these are essentially the same study, we take the geometric mean of

$$q_1^* = 1.9 \times 10^{-2}\ (\text{mg/kg/day})^{-1}$$

as the unit risk value.

In order to estimate a unit risk for a concentration of 1 ug/l drinking water, the following conversion is used.

$$1\ \text{mg/kg/day} \times 70\ \text{kg} \times 10^3\ \frac{\text{ug}}{\text{mg}} \times \frac{1\ \text{day}}{2\ \text{l}} = 3.5 \times 10^4\ \text{ug/l}$$

Therefore, the unit risk corresponding to 1 ug TCI per liter of water is

$$q_1^* = 1.9 \times 10^{-2}\ (\text{mg/kg/day})^{-1} \times \frac{1\ \text{mg/kg/day}}{3.5 \times 10\ \text{ug/l}} = 5.4 \times 10^{-7}\ (\text{ug/l})^{-1}$$

8.3.2.2 Inhalation--Two animal inhalation studies showed positive results in mice. These were the Henschler et al. (1980) study showing increased malignant lymphomas in females and the Bell et al. (1978) study showing increased hepatocellular carcinomas in males. As discussed in the qualitative section, however, both studies showed serious defects and are considered inadequate for calculating unit risk estimates. As an alternative the unit risk estimate based on both the NTP (1982) and NCI (1976) gavage studies can be used. That slope estimate must first be converted to units of ug/m^3 by the following conversion.

$$1\ \text{mg/kg/day} = 1\ \text{mg/kg/day} \times \frac{70\ \text{kg}}{\text{man}} \times \frac{\text{day}}{20\ \text{m}^3} \times \frac{10^3\ \text{ug}}{\text{mg}} = 3{,}500\ \text{ug/m}^3$$

or

$$1 \text{ ug/m}^3 = 2.86 \times 10^{-4} \text{ mg/kg/day}.$$

Data presented in the metabolism section show pulmonary absorption levels of TCI in humans as high as 75% of the inhaled amount. Assuming that 75% of inhaled TCI can be absorbed into the body, 1 ug/m^3 in the air would result in an absorbed effective dose of 2.15×10^{-3} mg/kg/day. The unit risk can then be estimated as

$$q_1^* = 1.9 \times 10^{-2} \text{ (mg/kg/day)}^{-1} \times 2.15 \times 10^{-4} \frac{\text{mg/kg/day}}{\text{ug/m}^3} = 4.1 \times 10^{-6} \text{ (ug/m}^3)^{-1}$$

8.3.2.3 Upper-limit Calculation Based on the Axelson et al. (1978) Study in Humans--Although the epidemiologic studies on TCI provide no evidence for its carcinogenicity, they can still be used to provide information on an upper limit of risk to be expected. The CAG adopts this procedure of using negative epidemiological studies for providing upper limits when animal studies are positive. Such use of negative human studies can modify estimates of potency from the positive animal studies.

The following 95% upper-limit estimates on relative risk are derived from data in Table 8-24 obtained by Axelson et al. (1978). These upper-limit estimates are calculated according to the following approach.

The 95% upper limit = u/expected number of cancer deaths (Table 8-27), where u is the solution to the sum of Poisson probabilities

$$\sum_{i=0}^{X} \frac{e^{-u} (u)^i}{i!} = 0.025$$

One might also interpret u as the upper limit of the number of workers with cancer deaths given the observed frequency, and X = number of observed workers with cancer deaths. Hence, the 95% upper-limit estimates for the cohort and each subcohort are as follows (Table 8-27).

TABLE 8-27. NUMBER OF WORKERS WITH CANCER DEATHS

Cohort	Observed X	Upper limit u	Expected if no effect	Relative risk 95% upper limit
Total cohort	11	19.7	14.5	1.4
Subcohort with 10 yr. latency (high and low exposure)	9	17.2	9.5	1.8
High exposure	3	8.7	1.8	4.8
Low exposure	6	13.3	7.7	1.7

These upper-limit relative risks can be used to provide estimates of the upper-limit probability of death from cancer due to a lifetime exposure to 1 ug/m^3 of TCI. This upper-limit probability estimate is 1.7×10^5 per ug/m^3 of TCI in air, and it is four times greater than the risk estimate based on the NCI (1976) and NTP (1982) carcinogenicity gavage bioassays. In showing these risk calculations below, the CAG emphasizes that:

1. This upper-limit value is derived from a negative epidemiologic study. Not only was there no specific target organ for cancer, but the total number of cancer deaths was less than expected.
2. Since there was no specific target organ, the upper-limit lifetime probability estimate is based on all cancer deaths. Since the lifetime probability of death from cancer to humans is 0.19 (based on 1977 U.S. death rates, race and sex combined), any even moderately conservative confidence limit will provide a large upper-limit risk.

The calculations are based on the subcohort data from the Axelson et al. (1978) study, specifically the low exposure group with at least a 10-year latency period. Table 8-27 projects an upper-limit relative risk of 1.7. Since the low exposure referred to those not exceeding a concentration of 100 mg trichloroacetic acid per ml in urine on the average, we equate this exposure to be not more than 30 ppm TCI in air. We use the 30 ppm as the 8-hour time-weighted average dose. This corresponds to 30 ppm x 10^3 x 1.2 x M_{TCI}/M_{air} = 30 x 10^3 x 1.2 x 131.4/28.8 = 1.64 x 10^5 ug/m^3. A lower dose estimate would provide an even higher upper-limit probability of death.

For years exposed, in the absence of any other information, we chose 10 years as a point assumed to be somewhere in the middle of the range.

With the above information we estimate the total lifetime dose of TCI to the low-dose exposure workers as:

$$\frac{(0 \text{ ppm} + 1.64 \times 10^5)}{2} \times \frac{240}{365} \times \frac{10}{70} = 7.7 \times 10^3 \text{ ug/m}^3 \text{ continuous}$$

where the 1.64 x 10^5 ug/m^3 TCI of the working day must be averaged with the 0 ug/m^3 of the nonworking day. We assume that one-half the air breathed during a day will be during working hours. Thus, the upper-limit estimate of the probability of death from 1 ug/m^3 TCI in air (continuous exposure) is estimated as:

$$P = \frac{\alpha\ (R - 1)d_1}{d_2} = \frac{0.19\ (1.7 - 1)1}{7.7 \times 10^3} = 1.7 \times 10^{-5} \text{ per ug/m}^3$$

where R = the relative risk, d_1 = 1 ppm, d_2 = dose of workers, and α = the background lifetime probability of death from all cancers.

Comparing this upper-limit estimate with that from the animal studies, the Axelson et al. study provides no evidence that human potency is less than that of animals.

8.3.3 Relative Potency

One of the uses of the concept of unit risk is to compare the relative potencies of carcinogens. To estimate relative potency on a per mole basis, the unit risk slope factor is multiplied by the molecular weight, and the resulting number is expressed in terms of $(\text{mg/Mol/kg/day})^{-1}$. This is called the relative potency index.

Figure 8-15 is a histogram representing the frequency distribution of potency indices of 54 chemicals evaluated by the CAG as suspect carcinogens. The actual data summarized by the histogram are presented in Table 8-28. Where human data are available for a compound, they have been used to calculate the index. Where no human data are available, animal oral studies and animal inhalation studies have been used in that order. Animal oral studies are selected over animal inhalation studies because most of the chemicals have animal oral studies; this allows potency comparisons by route.

The potency index for TCI based on mouse hepatocellular carcinomas in the NTP and NCI gavage studies is 5.8×10^{-1}. This is derived as follows: the mean slope estimate from both studies, 1.9×10^{-2} $(\text{mg/kg/day})^{-1}$, is multiplied by the molecular weight of 131.4 to give a potency index of 2.5×10^{0}. Rounding off to the nearest order of magnitude gives a value of 10^{0}, which is the scale presented on the horizontal axis of Figure 8-15. The index of 2.5 is among the least potent of the 54 suspect carcinogens, ranking in the lowest quartile. Ranking of the relative potency indices is subject to the uncertainty of comparing estimates of potency of different chemicals based on different routes of exposure for different species, using studies of different quality. Furthermore, all the indices are based on estimates of low-dose risk using linear extrapolation from the observational range. Thus, these indices are not valid for the comparison of potencies in the experimental or observational range if linearity does not exist there.

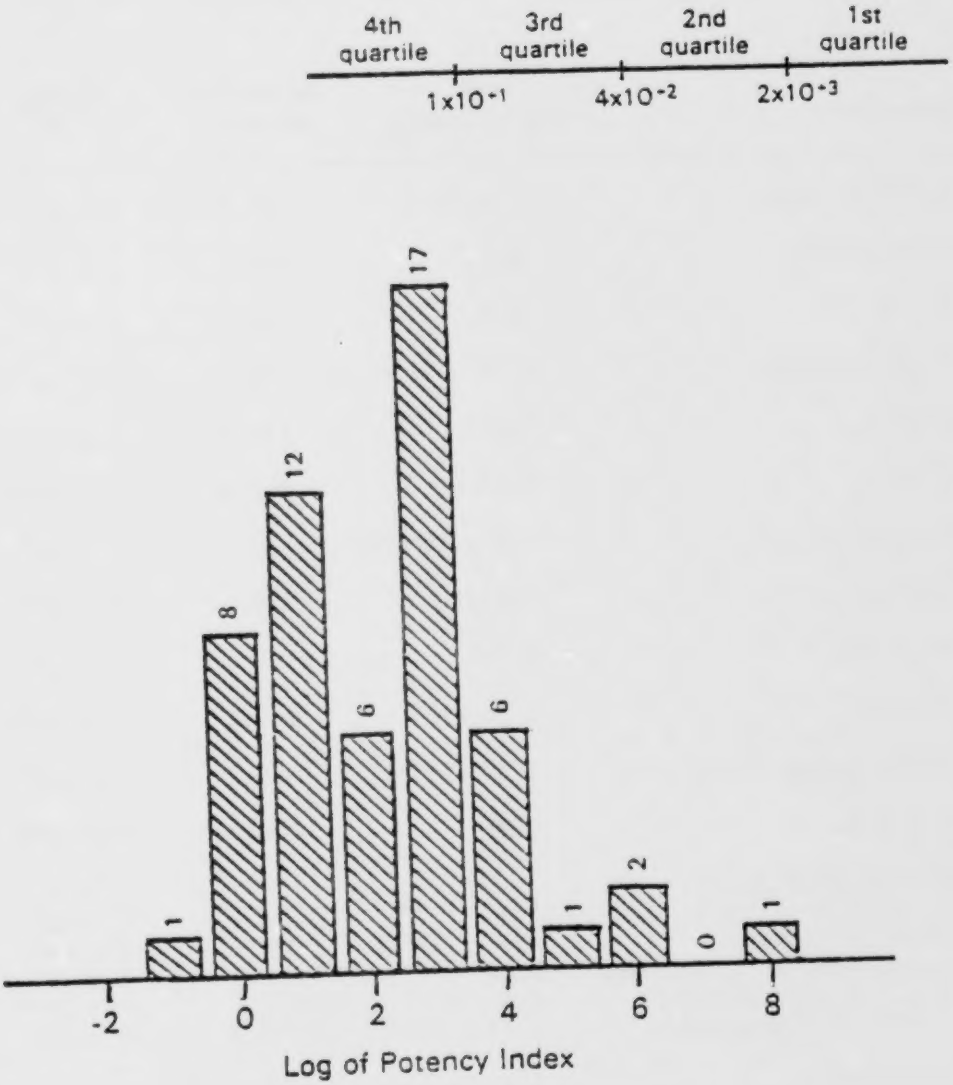


Figure 8-15. Histogram representing the frequency distribution of the potency indices of 54 suspect carcinogens evaluated by the Carcinogen Assessment Group.

TABLE 8-28. RELATIVE CARCINOGENIC POTENCIES AMONG 54 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS[1,2,3]

Compounds	Slope $(mg/kg/day)_{-1}$	Molecular weight	Potency index	Order of magnitude (log_{10} index)
Acrylonitrile	0.24(W)	53.1	$1 \times 10^{+1}$	+1
Aflatoxin B_1	2924	312.3	$9 \times 10^{+5}$	+6
Aldrin	11.4	369.4	$4 \times 10^{+3}$	+4
Allyl Chloride	1.19×10^{-2}	76.5	9×10^{-1}	0
Arsenic	15(H)	149.8	$2 \times 10^{+3}$	+3
B[a]P	11.5	252.3	$3 \times 10^{+3}$	+3
Benzene	5.2×10^{-2}(W)	78	4×10^{0}	+1
Benzidene	234(W)	184.2	$4 \times 10^{+4}$	+5
Beryllium	4.86	9	$4 \times 10^{+1}$	+2
Cadmium	6.65(W)	112.4	$7 \times 10^{+2}$	+3
Carbon Tetrachloride	1.30×10^{-1}	153.8	$2 \times 10^{+1}$	+1
Chlordane	1.61	409.8	$7 \times 10^{+2}$	+3
Chlorinated Ethanes				
1,2-dichloroethane	6.9×10^{-2}	98.9	7×10^{0}	+1
hexachloroethane	1.42×10^{-2}	236.7	3×10^{0}	0
1,1,2,2-tetrachloroethane	0.20	167.9	$3 \times 10^{+1}$	+1
1,1,1-trichloroethane	1.6×10^{-3}	133.4	2×10^{-1}	-1
1,1,2-trichloroethane	5.73×10^{-2}	133.4	8×10^{0}	+1
Chloroform	7×10^{-2}	119.4	8×10^{0}	+1
Chromium	41	104	$4 \times 10^{+3}$	+4
DDT	8.42	354.5	$3 \times 10^{+3}$	+3
Dichlorobenzidine	1.69	253.1	$4 \times 10^{+2}$	+3
1,1-dichloroethylene	1.47×10^{-1}(I)	97	$1 \times 10^{+1}$	+1
Dieldrin	30.4	380.9	$1 \times 10^{+4}$	+4

(continued on the following page)

TABLE 8-28. (continued)

Compounds	Slope (mg/kg/day)$_{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
Dinitrotoluene	0.31	182	6 x 10^{+1}	+2
Diphenylhydrazine	0.77	180	1 x 10^{+2}	+2
Epichlorohydrin	9.9 x 10^{-3}	92.5	9 x 10^{-1}	0
Bis(2-chloroethyl)ether	1.14	143	2 x 10^{+2}	+2
Bis(chloromethl)ether	9300(I)	115	1 x 10^{+6}	+6
Ethylene Dibromide (EDB)	8.51	187.9	2 x 10^{+3}	+3
Ethylene Oxide	0.63(I)	44.0	3 x 10^{+1}	+1
Formaldehyde	2.14 x 10^{-2}(I)	30	6 x 10^{-1}	0
Heptachlor	3.37	373.3	1 x 10^{+3}	+3
Hexachlorobenzene	1.67	284.4	5 x 10^{+2}	+3
Hexachlorobutadiene	7.75 x 10^{-2}	261	2 x 10^{+1}	+1
Hexachlorocyclohexane				
technical grade	4.75	290.9	1 x 10^{+3}	+3
alpha isomer	11.12	290.9	3 x 10^{+3}	+3
beta isomer	1.84	290.9	5 x 10^{+2}	+3
gamma isomer	1.33	290.9	4 x 10^{+2}	+3
Methylene Chloride	6.3 x 10^{-4}	84.9	5 x 10^{-2}	-1
Nickel	1.15(W)	58.7	7 x 10^{+1}	+2
Nitrosamines				
Dimethylnitrosamine	25.9(not by q_1^*)	74.1	2 x 10^{+3}	+3
Diethylnitrosamine	43.5(not by q_1^*)	102.1	4 x 10^{+3}	+4
Dibutylnitrosamine	5.43	158.2	9 x 10^{+2}	+3
N-nitrosopyrrolidine	2.13	100.2	2 x 10^{+2}	+2
N-nitroso-N-ethylurea	32.9	117.1	4 x 10^{+3}	+4
N-nitroso-N-methylurea	302.6	103.1	3 x 10^{+4}	+4
N-nitroso-diphenylamine	4.92 x 10^{-3}	198	1 x 10^{0}	0
PCBs	4.34	324	1 x 10^{+3}	+3

(continued on the following page)

TABLE 8-28. (continued)

Compounds	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude ($\log_{10}$ index)
Phenols 2,4,6-trichlorophenol	1.99×10^{-2}	197.4	4×10^{0}	+1
Tetrachlorodioxin	4.25×10^{5}	322	$1 \times 10^{+8}$	+8
Tetrachloroethylene	3.5×10^{-2}	165.8	6×10^{0}	+1
Toxaphene	1.13	414	$5 \times 10^{+2}$	+3
Trichloroethylene	1.9×10^{-2}	131.4	2.5×10^{0}	0
Vinyl Chloride	1.75×10^{-2}(I)	62.5	1×10^{0}	0

Remarks:

1. Animals slopes are 95% upper-limit slopes based on the linearized multi-stage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear non-threshold model.

2. The potency index is a rounded-off slope in $(mMol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

3. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

8.4 SUMMARY AND CONCLUSIONS

8.4.1 Qualitative Asssessment

8.4.1.1 Animal Studies--In a 90-week carcinogenicity bioassay with male and female B6C3F1 mice given estimated maximally (2,339 mg/kg in males and 1,739 mg/kg in females) and one-half maximally tolerated doses of technical grade, epoxide-stabilized TCI in corn oil daily, 5 days/week, for 78 weeks, statistically significant increases in the incidence of hepatocellular carcinomas in male and female mice compared to corn oil controls were found [National Cancer Institute (NCI) 1976]. Statistically significant increases in hepatocellular carcinoma incidence in treated male and female mice compared to vehicle-control mice were also observed in a repeat of the above carcinogenicity bioassay in which male and female B6C3F1 mice were treated with purified TCI, containing no detectable epoxides, in corn oil by gavage at a dose of 1,000 mg/kg/day, 5 days/week, for 103 weeks [National Toxicology Program (NTP) 1982]. In effect, this repeat study indicated that epoxide stabilizers were not necessary factors in the TCI-induced increases in hepatocellular carcinoma incidence in male and female B6C3F1 mice under the conditions of these bioassays. It is noted that spontaneous hepatocellular tumor formation is a characteristic of the B6C3F1 mouse strain.

A 110-week carcinogenicity study on male and female Osborne-Mendel rats given technical grade, epoxide-stabilized TCI in corn oil by gavage at estimated maximally (1,097 mg/kg) and one-half maximally tolerated doses daily, 5 days/week, for 78 weeks, was negative for carcinogenicity; however, this study may be inconclusive because of high mortality in the treatment groups (NCI 1976). In a carcinogenicity study on male and female Fischer 344 rats treated with purified TCI, containing no detectable epoxides, in corn oil by gavage at estimated maximally (1,000 mg/kg) and one-half maximally tolerated doses for 103 weeks, a small incidence of renal adenocarcinomas in high-dose males at terminal sacrifice (3/16) was calculated to be a statistically significant increase over that in matched vehicle treated controls (0/33) by life table and incidental tumor tests, which adjust for mortality, but not by the Fisher Exact Test (NTP 1982). However, the NTP, sponsor of the study, has concluded that this study is inadequate to evaluate the presence or absence of a carcinogenic response to TCI. Under the conditions of this study in Fischer 344 rats, there was a statistically significant increase in mortality in treated males and 2, 6, and 20% of the matched vehicle-control, low-dose, and high-dose males, respectively, were accidentally killed during the study. Toxic nephrosis characterized as cytomegaly was commonly found in the treated male and female Fischer 344 rats, with a stronger effect evident in males. A carcinogenicity study of purified TCI administered by gavage in olive oil to male and female Sprague-Dawley rats daily, 4 or 5 days/week for 52 weeks, at doses of 250 or 50 mg/kg followed by lifetime observation was negative, but the 52-week exposure period was below potential lifetime exposures for these animals (Maltoni 1979).

Inhalation exposure of male Han:NMRI mice, male and female Han:Wist rats, and male and female Syrian hamsters to purified TCI vapor at levels of 100 ppm and 500 ppm 6 hours/day, 5 days/week, for 78 weeks followed by lifetime observation did not induce a carcinogenic effect (Henschler et al. 1980). Overt toxicity of TCI was not evident in rats and hamsters, which suggests that testing at higher levels of TCI was possible in this study, but mortality was greater in male mice compared to controls. A statistically significant increase in malignant lymphoma incidence was found in low-dose and high-dose female Han:NMRI mice compared to controls in this study; however, the spontaneous incidence of malignant lymphomas in controls was high (30%), and it was postulated in the study report that the increased incidence of malignant lymphomas in female mice could have been a result of an immunosuppressive response capable of enhancing susceptibility to tumor induction by specific, mostly inborn, viruses (Henschler et al. 1980).

A chronic inhalation study of technical grade, epoxide-stabilized TCI as a vapor was done in which male and female Charles River rats and male and female B6C3F1 mice were exposed to nominal levels of TCI at 100, 300, and 600 ppm 6 hours/day, 5 days/week for 24 months (Bell et al. 1978). An audit of liver pathology revealed no carcinogenic effect in rats and a dose-related increase in the incidence of hepatocellular tumors in male and female B6C3F1 mice; however, this study is weakened by wide variability in the actual TCI exposure levels and lack of a concurrent matched control group of mice.

A series of studies done to evaluate the carcinogenic potential of purified TCI in lifetime treatment studies with female ICR/Ha Swiss mice included an initiation-promotion study in which a single topical application of 1 mg TCI in acetone was followed by lifetime repeated topical applications with the promoter phorbol myristate acetate; a study of complete carcinogenicity with repeated topical applications of 1 mg TCI in acetone; a study with once weekly subcutaneous injections of 0.5 mg TCI in trioctanoin; and a study in males as well as females with once-weekly gavage administrations of 0.5 mg TCI in trioctanoin (Van Duuren et al. 1979). Complete carcinogenic and initiating activities for TCI were not found in these studies. The dose used for topical application was below that estimated as maximally tolerated, but the authors concluded that no activity or low activity of chloroolefins tested for carcinogenicity on mouse skin would be expected because of a relatively low level of epoxidizing enzymes in mouse skin. More than once-weekly dosing by gavage and subcutaneous administration could have permitted a broader evaluation of TCI carcinogenicity.

Carcinogenic activity for TCI was not observed in several animal studies designed for an evaluation of general toxicity; however, these studies involved small groups and durations of treatment and observation that were less than the lifetime of the animals or, in one study, a duration that was not specified. Exposure of newborn Wistar rats for 10 weeks to 2,000 ppm TCI or vinyl chloride, 8 hours/day, 5 days/week induced ATPase-deficient foci, reported as premalignant lesions, in liver only in rats exposed to the latter agent (Laib et al. 1978, 1979); however, while only vinyl chloride was effective with this exposure duration, it is not certain whether longer exposure to TCI could ultimately have induced similar foci.

The carcinogenic potential of TCI oxide, a putative direct-acting metabolite of TCI, has also been evaluated in lifetime treatment studies with female ICR/Ha Swiss mice (Van Duuren et al. 1983). These studies included an initiation-promotion study in which a single topical application of 1 mg TCI oxide in acetone was followed by lifetime repeated topical applications with phorbol myristate acetate; a study of complete carcinogenicity with repeated topical applications of 2.5 mg TCI oxide in acetone; and a study with once-weekly subcutaneous injections of 0.5 mg TCI oxide in trioctanoin. These studies were negative for initiating activity and complete carcinogenic activity at estimated maximum tolerated doses.

8.4.1.2 Cell Transformation Studies--Cell transformation activity by TCI, at minimally toxic doses, and 3-methylcholanthrene, a positive control agent, was found in an in vitro study with Fischer rat embryo cells (F1706, subculture 108) containing genetic information of the Rauscher leukemia virus (Price et al. 1978). Cell transformation has been developed as an endpoint for predicting carcinogenic activity; however, it is not certain whether these agents elicited transformation in this Fischer rat cell line through an action on the host cells, their integrated oncornavirus, or both.

Exposure of baby Syrian hamster kidney (BHK-211Cl 13) cells in the presence of rat liver microsomes in vitro to TCI solution in DMSO at doses including those producing toxicity did not induce cell transformation, whereas vinyl chloride induced cell transformation when administered as a gas at levels including those producing toxicity, but not as a solution in DMSO tested at levels where toxicity was not clearly evident (Styles 1979). A broader evaluation could have been made with an additional test where cells were exposed to TCI as a vapor.

Cell transformation was induced by high concentrations (>1 mM) of TCI oxide capable of producing dose-related cytotoxicity in cultures of Syrian hamster embryo cells. The transformed colonies in this system were similar to those induced by benzo[a]pyrene and other chloroalkene epoxides (DiPaolo and Doniger 1982).

8.4.1.3 Human Studies--Three cohort studies of workers exposed to TCI did not find that these workers experienced an excess risk of cancer (Axelson et al. 1978, Tola et al. 1980, and Malek et al. 1979). Each of these studies, however, suffered from one or more of the following deficiencies: small sample size, lack of analysis by tumor site, problems with exposure definition, and problems with length of exposure.

Paddle (1983) found that none of the primary liver cancer cases residing in an area near an industrial plant that produced TCI had been employed by the plant. This study had several deficiencies, and there are also limitations in the author's description of the study which make it difficult to evaluate. No controls were used in the study, and the author provided no indication of the probability of finding a liver cancer case who had been an employee of the plant and had been exposed to TCI. The author provided no information on the dates or lengths of exposure of TCI workers or on whether the employee personnel lists included retirees only or all workers who had ever worked at the plant. Furthermore, persons with liver cancer who did not live in the area of the plant or who had moved from the area were excluded from the study by study definition.

Three proportionate mortality studies of dry-cleaning workers found some excesses for certain types of cancer deaths (Blair et al. 1979, Katz and Jowett 1981, and (Asal 1983, personal communication), and one of the studies (Blair et al. 1979) found an excess for deaths from all malignant neoplasms. None of these studies, however, identified what dry-cleaning agent or agents were used by the workers. Thus, any conclusions from these studies regarding an association of TCI with death from cancer are obviously limited.

A case-control study of pancreatic cancer cases found a statistically significant association between pancreatic cancer and the drinking of caffeine-free coffee (Lin and Kessler 1981). However, it is not known whether the decaffeinating agent for the coffee was TCI, or, if it was, whether any TCI was actually consumed by those who drank the decaffeinated coffee. An association was also found in this study between pancreatic cancer and occupational exposure in the dry-cleaning industry or occupational exposure to gasoline (service stations and garages). It is not known, however, how many of the pancreatic cancer cases involved dry-cleaning exposure and how many involved gasoline exposure. Secondly, the dry-cleaning agent used by the dry-cleaning workers was not identified. A kidney cancer case-control study found an association between kidney cancer and employment as a dry-cleaner (Asal 1983, personal communication). Again, however, the dry-cleaning agent used by the dry-cleaning workers was not identified.

In summary, there is no human evidence that TCI is a carcinogen. Although some studies have found an association between cancer and employment in the dry-cleaning industry, none of these studies indicated that TCI was the dry-cleaning agent that was used.

8.4.1.4 Conclusions--Evidence for the carcinogenicity of TCI in experimental animals includes increases in the incidences of hepatocellular carcinoma in male and female B6C3F1 mice, malignant lymphoma in female Han:NMRI mice, and renal adenocarinoma in male Fischer 344 rats. However, as discussed in this document, the inhalation study in B6C3F1 mice was weakened by deficiencies in its conduct; there was a 30% incidence of spontaneous lymphoma in control mice and the possibility of an indirect effect in treated mice in the inhalation study in Han:NMRI mice; and the gavage treatment study in Fischer 344 rats was concluded to be inadequate for a judgment of TCI carcinogenicity by the study's sponsor (NTP) because of experimental deficiencies. The gavage treatment study of purified TCI in male and female B6C3F1 mice was done with basically the same experimental design as the gavage treatment study of technical grade TCI in male and female B6C3F1 mice to evaluate the necessity of epoxide stabilizers in TCI in the induction of hepatocellular carcinomas.

Cell transformation activity by TCI in Fischer rat embryo cells containing rat oncornavirus was found, but it is not certain whether the effect could have occurred in the host genetic material, the oncornavirus, or both. As concluded in this document, currently available data provide suggestive evidence that commercial grade TCI is weakly active as an indirect mutagen, and data on pure TCI do not allow a conclusion to be drawn about its mutagenic potential; although, if it is mutagenic, the available data suggest that pure TCI would be a very weak indirect mutagen. As discussed in the metabolism chapter herein, there is evidence that TCI metabolite(s) can react with cellular protein macromolecules *in vivo* and *in vitro* and with exogenous DNA and RNA *in vitro*, whereas a study of purified radiolabeled TCI binding to DNA in liver *in vivo* in male BC63F1 mice given a gavage dose of 1,200 mg/kg of TCI-^{14}C (>500 uCi/animal) suggested a low order of binding [maximum of 0.62 (± 0.42) alkylated bases/10^6 nucleotides]. Cell transformation with high doses of TCI oxide, a putative direct-acting carcinogenic metabolite of TCI, has been observed *in vitro*; however, animal studies of initiating and complete carcinogenic potential of TCI oxide were negative.

TCI is positive for the induction of malignant tumors of the liver in both male and female B6C3F1 mice. This constitutes a signal that TCI might be a carcinogen for humans. The positive lymphoma response in females of the Han:NMRI mouse strain does qualify as a separate strain, but that result is only suggestive of a carcinogenic response because it occurs only in females, has a high spontaneous rate, and has been found to involve an indirect mechanism of action. It should be recognized that there is a substantial body of opinion in the scientific community to the effect that the mouse liver overreacts to chlorinated organic compounds in contrast to that of the rat, and that the induction of liver cancer in the mouse represents only a promoting action for spontaneous liver tumors, which normally occur with substantial incidence. Furthermore, this promoting action might be related to liver damage associated only with high-level exposures to chlorinated agents such as TCI. However, the evidence is inconclusive either for this restrictive position on the mouse liver carcinogenic response to chlorinated organics, or to the position that the mouse liver is as good as any other mammalian indicator of carcinogenicity for these compounds. The kidney tumor results in the NTP rat study are not strong indications of a response in a second species because of the small number of animals responding (3 of 49 animals) and because of the high mortality, although the statistical significance after mortality corrections suggests that a carcinogenic effect may be taking place. The classification of the TCI carcinogenicity results under the criteria of the International Agency for Research on Cancer (IARC) could be either "sufficient" or "limited" depending on the differing current scientific views about the induction of liver tumors in mice by chlorinated organic compounds. Since there are no adequate epidemiological data in humans, the overall ranking of TCI under the criteria of the IARC could be either Group 2B or Group 3, i.e., the more conservative scientific view would regard TCI as a probable human carcinogen, but there is considerable scientific sentiment for regarding TCI as an agent that cannot be classified as to its carcinogenicity for humans.

Additional ongoing carcinogenicity studies on purified TCI (no detectable epoxides) include those sponsored by the NTP with lifetime gavage treatment in four strains of rats (Osborne-Mendel, Marshall 540, August 28807, and ACI); those performed in the laboratories of Dr. C. Maltoni with lifetime inhalation exposure in BC63F1 mice, Swiss mice, and Sprague-Dawley rats; and those performed in the laboratories of Dr. D. Henschler with gavage treatment in ICR/Ha Swiss mice.

8.4.2 Quantitative Assessment

None of the epidemiologic studies cited above provide positive evidence from which to estimate a unit risk for exposure to TCI. As an alternative, an upper-limit risk has been estimated from one negative study for comparison with the two positive animal gavage studies. This upper-limit risk for air of 1.7×10^{-5} per ug/m^3 based on the human study is approximately four times greater than the estimate based on the mean of the two gavage studies, $q_1^* = 4.1 \times 10^{-6} (ug/m^3)^{-1}$, both of which showed hepatocellular carcinomas in mice. Hepatocellular carcinomas were also present in mice in one inhalation study, but this study displayed sufficient defects to exclude its use for a unit risk estimate. The results from the gavage studies were also used to estimate the unit risk from 1 ug/l of drinking water; this value was $q_1^* = 5.4 \times 10^{-7}$ $(ug/l)^{-1}$. The relative potency of TCI is 2.5, ranking it in the lowest quartile of 54 chemicals which the CAG has evaluated as suspect carcinogens. Furthermore, these estimates are valid upper limits only under the assumption that TCI is a potential human carcinogen. As discussed in the qualitative section, there is only limited evidence to support that assumption.

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